The reason for this being that the whole course of blue tongue averages 14 days, and this period must, therefore, be allowed for the reaction consequent on the vaccine before immunity is finally established. An animal already suffering from the disease at the date of vaccination would probably die within 9 days.

Deaths occurring between the 10th and 14th days are not considered as a result of natural infection, and the vaccine is probably held responsible. At the same time I must point out that of the 299 sheep vaccinated at this station, none died, but when the vaccine is used in practice on thousands of animals, some deaths are certain to occur. Mortality after the reaction has finished (that is to say, from the 14th day onwards) have been considered as relapses (breakdowns in immunity).

As in many instances farmers did not inoculate their whole flocks, statistics were also collected regarding the mortality amongst the non-vaccinated animals, and, for the purposes of comparison, have been embodied in the following return :—

RETURNS OF MORTALITY AMONGST VACCINATED SHEEP AS COMPARED WITH MORTALITY OF NON-VACCINATED SHEEP RUNNING ON THE SAME FARM.

DISTRICT.	VACCINATED SHEEP.				NON-VACCINATED SHEEP.		PER CENT. OF DEATHS AMONGST	
	No. Vaccinated.	No. which Died within. 1—9 Days.	No. which Died within. 10—14 Days.	No. which Died after 14 Days.	Number.	No. which Died.	Vaccinated Animals.	Non-Vaccinated.
							Per cent.	Per cent.
Ermelo ...	1,906	12	17	2	3,228	336	0·9	10·
Heidelberg ...	23	1	0	2	3,542	283	0	8·
Middelburg ...	142	39	6	0	2,204	599	3·0	27·
Lydenburg ...	966	0	1	3	2,289	200	0·1	9·
Marico ...	36	1	0	0	400	16	0	4·
Waterberg ...	1,200	0	0	0	Not	stated.	0	—
Rustenburg ...	11	0	0	0	,,	,,	0	—
Pretoria ...	1,065	19	1	0	,,	,,	0·1	—
Barberton ...	10	0	0	0	130	Nil	0	0
Standerton ...	106	2	1	0	4,425	383	0·9	9·
Volksrust ...	10	0	0	0	—	—	0	—
	5,875	74	26	7	16,218	1,817	0·4	11·0

Naturally these figures only represent a small minority of the sheep in the Transvaal, and the number vaccinated during the season, but the return is accurate as regards the statistics at my disposal, and the results may safely be considered as typical for the Colony. The percentage of deaths due to the vaccination is 0.4 per cent., and relapses amount to 0.1 per cent.

These results show very clearly the advantage vaccinated sheep have over susceptible animals.

TRANSVAAL

DEPARTMENT OF AGRICULTURE.

REPORT

— OF THE —

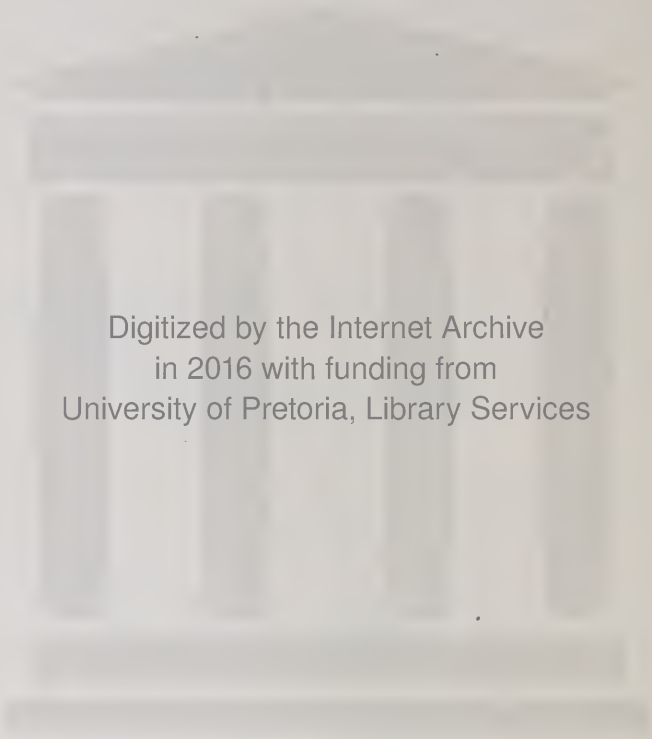
Government Veterinary Bacteriologist

— FOR THE —

YEAR 1907-08.

PRETORIA:
GOVERNMENT PRINTING AND STATIONERY OFFICE.
1909.

5434—25/8/09—1,000.



CONTENTS.

Division of Veterinary Science,

Pretoria,

1st August, 1909.

To the Director of Agriculture.

Sir,

I have the honour to submit herewith a detailed summary of the experimental work carried out at the Bacteriological Laboratory during the year ended the 30th June, 1908.

My general remarks with regard to the work performed by this Division have already been submitted to you and appear in the Annual Report of the Department of Agriculture for the year 1907–08.

I have the honour to be,

Sir,

Your obedient Servant,

A. THEILER,

Government Veterinary Bacteriologist,

PART I.

INVESTIGATIONS INTO SOUTH AFRICAN DISEASES.

By Dr. Arnold Theiler, C.M.G.,
Government Veterinary Bacteriologist.

A.—THE IMMUNITY OF CATTLE INOCULATED WITH PIROPLASMA MUTANS.

In Nelspruit, on the very place where our experiments in 1903 were conducted with East Coast fever, the proprietor introduced some ten calves from the high veld early in 1908; the majority of them died, and microscopical examination of smears showed the presence of small piroplasms to such an extent that for a short time the diagnosis was doubtful, and for safety's sake East Coast fever had to be declared.

An investigation was immediately made, and it was then clearly proved that the calves were susceptible to a *Piroplasma mutans* infection, and *Piroplasma parvum* had to be excluded. The fact that a strong infection of *Piroplasma mutans* was present suggested the idea of seeing to what extent cattle introduced on this particular farm would contract the infection. Arrangements were made with the proprietor, and the cattle to be exposed were selected from our stock and divided into five lots, namely: (1) Animals immune against redwater; (2) animals immune against redwater and *Piroplasma mutans*; (3) knowing that the farm was also infected with heartwater, animals immune against redwater and heartwater; (4) animals immune against redwater, heartwater, and *Piroplasma mutans*; and (5) with animals not immunised in any way, to serve as controls.

Experiment No. 1.

With animals immune against redwater.

1. *Bull* 309.—Born in the Transvaal, and running on infected veld for two years.

   Note.—This animal is immune against redwater.

   Exposed at Nelspruit on the 13th March, 1908.

   Slight reaction from the 15th day, lasting four days, during which time *Piroplasma mutans* in rare numbers were noted—1st April, 1908. A secondary reaction noted from the 25th day, touching 105·4, six days later and subsiding on the 28th day.

   Discontinued on the 11th June, 1908.

2. *Ox* 342.—Born on the station in November, 1905, and had been running on redwater veld for two years.

NOTE.—Immune against redwater.

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 13th day, *Piroplasma mutans* in rare numbers and the lesions of a slight poikilocytosis being present five days later.

Secondary reaction from the 7th April, 1908, lasting for thirteen days, blood examinations on the 9th April, 1908, being negative. With the exception of a sharp rise from a morning record of 98 on the 16th May, 1908, to an evening reading of 105 three days later, no further symptoms were noted.

Discontinued on the 14th June, 1908.

Results.—Two animals immune against redwater alone, and exposed to natural infection, both showed slight reactions, accompanied with *Piroplasma mutans.*

EXPERIMENT No. 2.

With animals immune against redwater and Piroplasma mutans.

1. *Ox* 380.—Imported from Cape Colony.

Injected on the 6th June, 1908, subcutaneously with 5 c.c. blood of calf 378 (immune against redwater and *Piroplasma mutans*), developed a *Piroplasma mutans* reaction, accompanied with parasites.

Injected on the 4th November, 1906, with 5 c.c. heifer No. 316 (an animal infected with *Piroplasma mutans* and ordinary redwater); *Piroplasma mutans* again noted, together with a reaction, but this was probably caused as a result of vaccination with lymph. (See Annual Report, 1906-07, page 57).

NOTE.—Immune against redwater and *Piroplasma mutans.*

Exposed at Nelspruit on the 13th March, 1908.

Typical temperature reaction from the 12th day, reaching a maximum of 105·6 eleven days later, and regaining normal on the 16th April, 1908.

Piroplasma mutans and a slight poikilocytosis were noted on the 1st and 4th April, 1908; the latter lesion also being present on the 31st March, 1908, and 9th April, 1908.

Discontinued on the 14th June, 1908.

2. *Heifer* 400.—Imported from Aliwal North.

Injected on the 16th October, 1906, subcutaneously with 5 c.c. calf 397 (an animal immune against ordinary redwater), a reaction followed, accompanied with poikilocytosis.

On the 18th December, 1906, used in experiment with English redwater (see Annual Report, 1906-07, page 65); negative results.

Injected on the 30th January, 1908, with 10 c.c. blood of calf 425 (this animal had been inoculated with blood obtained from a calf in Zeerust which showed *Piroplasma mutans* in its blood).

Reaction from the 13th day, accompanied with *Piroplasma mutans.*

NOTE.—This animal is immune against redwater and *Piroplasma mutans.*

Exposed at Nelspruit on the 13th March, 1908.

Slight reaction from the 15th day, but with no high evening temperature record, the morning reading being below 101 during the following seventeen days; microscopical examinations negative.

Discontinued on the 14th June, 1908.

3. *Heifer* 418.—Imported from Cape Colony.

Injected on the 13th February, 1906, with English redwater (see Annual Report, 1906–07, page 66); negative results.

Injected on the 26th March, 1907, with 10 c.c. blood of heifer 425 (an animal containing *Piroplasma bigeminum* and *mutans* in its blood—see Annual Report, 1906–07, page 60). Irregular reaction accompanied with *Piroplasma mutans*.

NOTE.—This animal is immune against redwater and *Piroplasma mutans*.

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 11th day, temperature rising daily. On the 18th day the morning temperature recorded 104, and microscopical examination showed *Piroplasma mutans* in rare numbers. The following day the temperature dropped to a morning record of 102·2, rising to 105·6 in the evening; on microscopical examination slight poikilocytosis and *Piroplasma mutans* were noted; the same lesions were present the next day, and the animal died during the ensuing evening (2nd April, 1908) from heartwater.

4. *Heifer* 419.—Imported from Cape Colony.

Injected on the 13th December, 1906, with blood of heifer 425 (an animal containing *Piroplasma bigeminum* and *mutans* in its blood—see previous animal, heifer 418); reaction accompanied with *Piroplasma mutans*.

NOTE.—This animal is immune against redwater and *Piroplasma mutans*.

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 14th day, the temperature slowly rising for four days; slight poikilocytosis and *Piroplasma mutans* in rare numbers being noted on the 18th day; a sharp rise now followed, with an evening record of 106 on the 22nd day, remaining high for the three subsequent days; death from heartwater occurring on the morning of the 26th day—8th March, 1908—smears taken just previous to death showed slight poikilocytosis and *Piroplasma mutans*.

5. *Heifer* 421.—Imported from Cape Colony.

Injected on the 13th December, 1906, with English redwater (see Annual Report, 1906–07); negative results.

Injected on the 30th January, 1907, with 10 c.c. of blood of ox 126 (an animal immune against ordinary redwater); reaction with *Piroplasma bigeminum*.

Injected on the 8th March, 1907, with 10 c.c. heifer 409 (an animal containing *Piroplasma bigeminum* and *Piroplasma mutans* in its blood—see Annual Report, 1906–07, page 55); reaction with *Piroplasma bigeminum* and *Piroplasma mutans*.

NOTE.—This animal is immune against redwater and *Piroplasma mutans*.

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 15th day, a slight poikilocytosis and *Piroplasma mutans* being noted three days later; the temperature remained high for several days, and the same lesions were present on the 13th April, 1908—31st day. Normal record obtained from the 24th April, 1908, until the 15th May, 1908, when a sudden rise to 107 was noted in the evening, followed ten days later by another sharp rise to 106.

Discontinued on the 14th June, 1908.

6. *Heifer* 473.—Imported from Cape Colony.

Injected on the 7th November, 1907, with blood of ox No. 111, and showed *Piroplasma bigeminum* and *mutans*.

Note.—This animal is immune against redwater and *Piroplasma mutans.*

Exposed at Nelspruit on the 13th March, 1908.

Typical fever reaction from the 15th day, lasting for twenty-four days, during which time *Piroplasma mutans* was present on the 19th, 23rd, 26th, and 27th days, together with the lesions of a slight poikilocytosis; the latter being also apparent on the 29th and 31st day.

Discontinued on the 14th June, 1908.

7. *Ox* 479.—Imported from Cape Colony.

Injected on the 7th November, 1907, with 5 c.c. blood of ox 441 (see above); reaction followed, accompanied with *Piroplasma mutans* and poikilocytosis.

Note.—This animal is immune against redwater and *Piroplasma mutans.*

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 8th day, reaching 106 five days later, and 106·6 eight days later again, returning to normal on the 24th April, 1908—42nd day. Microscopical examination showed *Piroplasma mutans* and slight poikilocytosis on the 22nd, 23rd, 26th, 30th, and 31st days, the latter lesion being also present on the 27th and 29th days.

Discontinued on the 14th June, 1908.

8. *Ox* 480.—Imported from the Cape Colony.

Injected on the 11th November, 1907, with 5 c.c. ox 469, an animal infected with *Piroplasma mutans* and *bigeminum.*

Reaction followed, accompanied with a slight poikilocytosis.

Note.—This animal is immune against redwater and *mutans.*

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 15th day, rising slowly to a maximum evening temperature of 106 on the 30th day; microscopical examination showed slight poikilocytosis and *Piroplasma mutans* on the 26th, 27th, and 31st days; *Piroplasma bigeminum* appeared on the 29th day, and a slight poikilocytosis the following day. Temperature remained very irregular from the 17th April, 1908, occasionally reaching 105 and 106 in the evening.

Discontinued on the 14th June, 1908.

Results.—Eight animals immune against redwater and *Piroplasma mutans* showed reactions when exposed to natural infection, in seven of them accompanied with *Piroplasma mutans.*

Experiment No. 3.

With animals immune against redwater and heartwater.

1. *Ox* 262.—Imported from Cape Colony in September, 1904.

On the 7th November, 1904, infested with larval blue ticks from ox 347, suffering from redwater and spirillosis, and reinfested on the next four days. No reaction.

Injected on the 25th June, 1906, with 20 c.c. blood of goat 119, an animal affected with heartwater.

No reaction.

Note.—This animal is immune to redwater and heartwater.

Exposed at Nelspruit on the 13th March, 1908. Indications of a very slight reaction from the 3rd to 21st days.

Blood examinations negative. Rise of temperature from the 33rd day, with an evening record of 104 to 105 for two weeks.

Discontinued on the 14th June, 1908.

2. *Bull* 302.—Born and bred in the Transvaal.

Injected on the 6th June, 1906, subcutaneously with 20 c.c. blood of sheep 484, an animal infected with heartwater. No distinct reaction; the morning temperature remained about 98·4, rising to an evening record of 103 to 104. Exposed in a redwater infected area for three months.

Note.—This animal is immune against redwater and heartwater.

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 9th day, lasting eleven days, during which time slight poikilocytosis and *Piroplasma mutans* appeared. Morning and evening temperature remained normal.

Discontinued on the 14th June, 1908.

3. *Bull* 305.—Born and bred in the Transvaal.

Injected on the 6th June, 1906, intrajugularly with 20 c.c. blood of sheep 484, an animal infected with heartwater. Slight reaction from the 17th day; exposed to a redwater infected area during 1907.

Note.—This animal is immune against redwater and heartwater.

Exposed at Nelspruit on the 13th March, 1908.

Short rise noted from the 13th day, touching 105 three days later, and regaining normal on the 24th day. Typical fever reaction followed, touching 108 on the 10th of April (twenty-eight days after exposure); slight poikilocytosis and *Piroplasma mutans* in rare numbers noted two days previously and again on the 28th day. Temperature slowly fell, returning to normal on the 26th April, 1908, or forty-six days after exposure.

Discontinued on the 14th June, 1908.

Results.—Three animals immune against redwater and heartwater showed slight reactions when exposed to natural infection, accompanied in two instances with *Piroplasma mutans.*

Experiment No. 4.

With animals immune against redwater, heartwater, and Piroplasma mutans.

1. *Ox* 229.—Imported from Cape Colony early in 1903.

Injected on 3rd December, 1903, with blood containing *Piroplasma bigeminum* and *mutans*; slight primary and distinct secondary reaction followed; examination of blood on 25th February, 1904, showed the presence of small piroplasms.

Injected on 1st February, 1904, with blood of sheep 102 (suffering from heartwater); negative results.

During August, 1904, and the following months hyperimmunised with heartwater blood to the extent of 4 litres.

On the 24th May, 1906, infused with 1,000 c.c. blood of calf 323, suffering from heartwater and *Piroplasma mutans.*

Reaction from 15th day, lasting six days.

Note.—This animal is immune against *Piroplasma bigeminum, mutans,* and heartwater.

Exposed at Nelspruit on the 12th March, 1908; sharp rise on the 12th day, reaching 106 twenty-four hours later, and returning to normal on the 15th day. Blood examinations negative. Morning temperature remained at 101 for the next fourteen days, and from then recorded 98 to 99, with an evening record of 103. From the 15th May, the difference in the morning and evening temperature varied between 5° and 7°.

Discontinued on the 14th June, 1908.

2. *Ox* 244.—Imported from Cape Colony in July, 1904.

On the 30th July, 1904, infested with heartwater infected ticks.

Reaction from the 24th day. On the 25th October, 1904, hyperimmunised with heartwater blood.

Injected on the 24th May, 1906, with 1,000 c.c. blood of calf 323 (heartwater), an animal which also contained *Piroplasma bigeminum* and *mutans* in its blood. Slight reaction from the 15th day.

NOTE.—This animal is immune against *Piroplasma bigeminum*, *mutans*, and heartwater.

Exposed at Nelspruit on the 12th March, 1908.

Slight rise on the 13th day, reaching 104·4 twenty-four hours later and returning to normal on the 24th day. Blood examinations on the 18th and 19th days negative. Temperature remained normal, with a morning record of 98 to 99, and 102 or 103 F., in the evening.

Sharp rise lasting from the 31st May to the 6th of June, and reaching the maximum of 105 on the evening of 3rd June, 1908.

Discontinued on the 14th June, 1908.

3. *Ox* 269.—A Transvaal animal, purchased in August, 1904.

Injected on the 15th November, 1905, with 10 c.c. blood of ox 347, an animal infected with *Piroplasma bigeminum* and *mutans*.

No reaction, and no piroplasms noted.

On the 21st March, 1906, infused with blood of ox 361, an animal affected with heartwater. Reaction from the 7th day, lasting three weeks. On the 28th December, 1906, hyperimmunised with blood of ox 390, an animal infected with heartwater. No result.

NOTE.—This animal is immune against *Piroplasma bigeminum*, *mutans*, and heartwater.

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 14th day, reaching 106·2, three days later; microscopical examinations negative. Temperature fell on 4th April, 1908, and another reaction commenced three days later, lasting for a week.

Discontinued on the 14th June, 1908.

4. *Bull* 320.—Born in the Transvaal; had been running on redwater infected area for one year, and was in the low veld for two months.

Injected on the 24th July, 1906, with 10 c.c. blood of sheep Nos. 347, 349, and 381, these animals being infected with heartwater. Slight reaction from the 8th to 16th days.

Injected on the 24th July, 1906, with 20 c.c. goat 136 (goat 136 had been injected with blood of cow 364, an animal suffering from heartwater).

Reaction from the 16th day, lasting for nine days.

NOTE.—This animal is immune against redwater, heartwater, and also possibly against *Piroplasma mutans* (by reason of exposure in the low veld).

Exposed at Nelspruit on the 13th March, 1908.

Reaction noted from the 13th day, lasting nine days, but microscopical examinations gave negative results. Secondary reaction from the 7th April, 1908, lasting until the 21st April, 1908.

Discontinued on the 14th June, 1908.

5. *Ox* 337.—Born in Potchefstroom; had been running in redwater veld, and was also in the low country for two months.

Injected on the 24th July, 1906, intrajugularly with 20 c.c. goat 136 (this animal had been injected with blood of cow 364, infected with heartwater). Fever reaction from the 14th day, lasting for seven days.

Note.—This animal is immune against redwater, heartwater, and possibly also against *Piroplasma mutans* (by reason of exposure in low veld).

Exposed at Nelspruit on the 13th March, 1908.

Slight reaction from the 15th day, lasting until the 24th day, during which time *Piroplasma mutans* in rare numbers were noted on the 19th day. Secondary reaction from the 7th April, 1908, the temperature rising to 105 two days later—microscopical examinations being negative—slowly falling until the 20th April, 1908, when a sharp rise was noted, recording 106 in the evening three days later.

Discontinued on the 14th June, 1908.

Results.—Two animals immune against redwater, heartwater, and *Piroplasma mutans* all showed slight reactions, and in one case accompanied with *Piroplasma mutans*, but it is doubtful whether this animal was immune against the disease.

Experiment No. 5.

With susceptible animals.

1. *Ox* 547.—Imported from the Cape Colony.

Had not been previously injected, and served as one of the controls in the experiment.

Exposed at Nelspruit on the 13th March, 1908.

Reaction from the 8th day, rising to 107 on the 21st day, and followed by death from heartwater thirty-six hours later (4th April, 1908).

Microscopical examinations showed slight poikilocytosis and *Piroplasma mutans* on the 18th, 20th, and 22nd days, the former lesion being also noted on the 17th and 18th days. Blood taken at time of post-mortem examinations showed *Piroplasma mutans.*

2. *Ox* 548.—Imported from the Cape Colony.

This animal had not been injected previously, and served as the second control in the experiment.

Exposed at Nelspruit on the 13th March, 1908.

Typical reaction from the 3rd day, the temperature touching 107 on the 12th, 17th, and 18th days, falling for the next five days, followed by another rise to 106·8 on the 26th day; death from heartwater, complicated with redwater, occurred on the following morning. Slight poikilocytosis and *Piroplasma mutans* in rare numbers were noted on the 20th, 22nd, 23rd, and 26th days; *Piroplasma bigeminum* appeared on the 27th day.

Results.—The two control animals both died showing lesions of heartwater. In both cases *Piroplasma mutans* was present in the blood, and one animal's death was complicated with redwater.

Experiment No. 6.

With blood taken from animals exposed at Nelspruit and injected into susceptible sheep and goats.

Animals Nos. 479, 419, 480, 380, 337, 473, 305, 302, 342, and 400 were tapped during the reaction on 8th April, and, with the exception of Nos. 479 and 419, were again tapped on the 11th of April, 1908. The blood was immediately forwarded to Pretoria and injected into the following animals:—

(*A*) Injections on the 10th April, 1908, with blood collected on the 8th April, 1908.

"A." *Goat* 1363.—Injected with 5 c.c. blood of ox 479, not immune against heartwater.

Result.—Reaction followed, temperature recording 105·8 on the 19th day. Death from heartwater occurred on the 30th April, 1908, twenty days after injection.

" B." *Sheep* 426.—Injected with 5 c.c. blood of ox 419, not immune against heartwater.

Result.—Reaction from the 7th day, lasting seven days, followed by a doubtful heartwater reaction from the 28th to 36th day.

(*B*) The following animals received two injections, the first on the 10th April, 1908, with blood taken on the 8th April, 1908, and the second on the 13th April, 1908, with blood collected at Nelspruit on the 12th April, 1908 :—

" C." *Sheep* 611.—Injected with 5 c.c. blood of ox 480, not immune against heartwater, followed by a second injection of 5 c.c. of the same ox.

Result.—Reaction from the date of the second injection lasting fifteen days, followed immediately by a doubtful heartwater reaction lasting ten days.

" D." *Sheep* 660.—Injected with 5 c.c. blood of ox 380, not immune against heartwater ; injection repeated three days later.

Result.—Reaction nine days after first injection, lasting nine days, and followed immediately afterwards by a doubtful heartwater reaction lasting eleven days.

" E." *Sheep* 671.—Injected with 5 c.c. blood of ox 337, immune against heartwater, redwater, and *Piroplasma mutans* (?) ; injection repeated three days later.

Result.—Irregular atypical reaction for six days after first injection, followed by a slight reaction lasting twelve days ; not typical for heartwater.

" F." *Sheep* 678.—Injected with 5 c.c. blood of heifer 473, not immune to heartwater ; injection repeated three days later.

Result.—Very slight indication of a temperature reaction ; not typical for heartwater.

" G." *Sheep* 825.—Injected with 5 c.c. blood of bull 305, immune against heartwater ; injection repeated three days later.

Result.—Reaction from date of second reaction, lasting fourteen days, followed immediately by a doubtful heartwater reaction which continued for nine days.

" H." *Sheep* 934.—Injected with 5 c.c. blood of bull 302, immune against heartwater ; injection repeated three days later.

Result.—Irregular temperature for eight days after first injection, followed by a slight atypical reaction prolonged for about three weeks.

" I." *Sheep* 1146.—Injected with 5 c.c. blood of ox 342, not immune against heartwater ; injection repeated three days later.

Result.—Reaction from date of second injection, lasting fourteen days, followed immediately by a reaction not typical for heartwater, lasting about two weeks.

" J." *Sheep* 1149.—Injected with 5 c.c. blood of ox 479, not immune against heartwater ; injection repeated three days later.

Result.—No distinct fever reaction.

" K." *Sheep* 1234.—Injected with 5 c.c. blood of heifer 400, not immune against heartwater ; injection repeated three days later.

Result.—Slight reaction from fourth day after first injection, lasting eight days, followed by a doubtful heartwater reaction, continuing for about two weeks.

Results.—Of ten sheep and one goat injected with blood collected from the exposed cattle, all showed reactions, and the goat died of heartwater.

TABULATED ANALYSIS OF RESULTS.

No. of Animal.	Immuno against.	Result when exposed to infection at Nelspruit.	Results given by blood taken from these cattle when injected into sheep or goats.
309	Redwater	Slight reaction with *P. mutans*	Not tested.
342	,, ...	,, ,, ,,	Tested on sheep; reaction followed not typical for heartwater.
380	Redwater and *P. mutans*	Reaction with *P. mutans* ..	,, ,, ,,
400	,, ,,	Reaction	,, ,, ,,
418	,, ,,	Reaction with *P. mutans*, and died of heartwater	Not tested.
419	,, ,,	,, ,, ,,	Tested on sheep; reaction followed not typical for heartwater.
421	,, ,,	Reaction with *P. mutans* ..	Not tested.
473	,, ,,	,, ,, ..	Tested on sheep; reaction followed not typical for heartwater.
479	,, ,,	,, ...	Caused a goat to contract a heartwater reaction, and died from this disease; did not cause a reaction in sheep.
480	,, ,,	,, ,, ..	Tested on sheep; reaction followed not typical for heartwater.
262	Redwater and heartwater	Slight reaction	Not tested.
302	,, ,,	Slight reaction and *P. mutans*	Tested on sheep; reaction followed not typical for heartwater.
305	,, ,,	,, ,, ,,	,, ,, ,,
229	Redwater, heartwater, and *P. mutans*	Slight reaction	Not tested.
244	,, ,,	,, ,, ...	,,
269	,, ,,	,, ,, ...	,,
320	Redwater, heartwater, and *P. mutans* (?)	,, ,, ...	,,
337	,, ,,	Slight reaction and *P. mutans*	Tested on sheep; reaction followed not typical for heartwater.
547	Susceptible; control animal	Reaction with *P. mutans*, and died of heartwater	Not tested.
548	,, ,,	Reaction with *P. mutans* and *P. bigeminum*; died of heartwater complicated with redwater.	

RÉSUMÉ.

1. The two animals immune against redwater alone showed reactions when exposed, during which *Piroplasma mutans* was observed. This *Piroplasma mutans* infection must be considered to be a natural infection, but the reaction may not be due to *Piroplasma mutans* alone, but also to the undetermined disease alluded to later.

2. Two of the eight animals immune against redwater and *Piroplasma mutans* died; the post-mortem lesions corresponded to that of heartwater. The remaining six animals all showed reactions, accompanied with *Piroplasma mutans* in five cases.

3. The three animals immune against redwater and heartwater showed slight fever reactions when exposed, accompanied in two instances with *Piroplasma mutans*.

4. The five animals immune against redwater, *Piroplasma mutans*, and heartwater all showed slight reactions when exposed, and only one animal (337), of which there exists a doubt as to whether it was immune to *Piroplasma mutans*, showed this parasite. In the other animals the reaction did not cause the reappearance of *Piroplasma mutans*; in 337 it is probable that the reaction was either due to a relapse of heartwater or to some other agency.

5. Both control animals died, showing lesions of heartwater; both showed *Piroplasma mutans* in the blood, and one also showed an infection of *Piroplasma bigeminum* at post-mortem.

6. All the exposed animals showed reactions a certain time after exposure; these reactions cannot be determined with absolute certainty, although inoculation of blood collected during the reactions were made into various sheep. Only one goat contracted a typical heartwater reaction and died; the remainder —all sheep—showed reactions, but since none died, and heartwater is usually fatal for sheep, we must conclude that not all the reactions given by the exposed cattle were due to heartwater, but that there was some other agency responsible, of which we have no knowledge at the present time.

7. With regard to the reaction, which cannot be definitely determined, it must be remembered that the animals had been for a considerable length of time away from tick infection, or at least were exposed to a minimum tick infection, and running on veld in which the blue and red ticks were present, and the brown was hardly ever noticed. In the bushveld the bont and the brown tick preponderate, and it is possible that these are responsible for this fever.

Conclusions.

1. The exposure of animals immune against redwater in the low veld proved that this immunity protected against the redwater of that veld.

2. Animals immune against heartwater were protected against that disease in the low veld.

3. Animals which were only immune against redwater contracted a *Piroplasma mutans* infection when exposed in the low veld.

4. All the animals which were not immune against *Piroplasma mutans* contracted this infection when exposed in the low veld, but none died.

5. Of the two control animals which were not immune against any of the diseases both died; *Piroplasma mutans* was present in both cases, but the deaths were due to heartwater, and in one case complicated with redwater.

6. All the exposed animals showed reactions, due either to heartwater or to some other agency, and this reaction, in the majority of cases, caused an increase in the number of *Piroplasma mutans* present in the blood.

7. The animals which were immune against heartwater, *Piroplasma mutans*, and *Piroplasma bigeminum* showed a slight *Piroplasma mutans* infection, and also a slight reaction.

B.—THE INFLUENCE OF COLD ON TICKS AND PIROPLASMA PARVUM.

Shortly after the introduction of East Coast fever into the low veld of the Elands River Valley in the Eastern Transvaal, and before legislation prohibited the movement of cattle, in several instances infected herds were brought up from that district to the high veld. One particular case came under my

observation in the neighbourhood of Carolina. It was generally noted that directly after the introduction of sick cattle to the high veld, the infected cattle died out, and the remainder of the herd did not contract the disease, neither did the cattle which were grazing on areas over which the infected cattle must have dropped ticks. At that time it was not known that *Rhipicephalus evertsi* (the red tick) was a carrier of East Coast fever, and the observation was apparently explained since *Rhipicephalus appendiculatus* (the brown tick), which is the principal carrier of the disease, was not found amongst the surviving cattle at the time the examination was made and it was, therefore, concluded that *Rhipicephalus appendiculatus* could not live in the high veld.

Later investigations, however, proved that *Rhipicephalus appendiculatus* may live in the protected places in very high altitudes, such as dongas, but it is not present in large numbers. In 1906, Mr. Lounsbury, Government Entomologist of the Cape Colony, stated that *Rhipicephalus evertsi* was also a carrier of this disease, and in my previous Annual Report I was able to corroborate this statement, with the reservation that *Rhipicephalus evertsi* is not such a certain carrier of *Piroplasma parvum* as the brown tick, as in several instances I failed to infect *Rhipicephalus evertsi*.

Considering that *Rhipicephalus evertsi* is one of the most common ticks of the high veld, although it is not found in such large numbers as in the low veld, it became apparent that the disappearance of East Coast fever from the high veld had to be interpreted in a different way.

The influence of cold as a possible factor in the destruction of the virus within the tick suggested itself, and for this purpose, experiments were undertaken with *Rhipicephalus appendiculatus*.

In the first instance, it was surmised that on account of the considerable amount of liquid contained in engorged nymphae, this would be the stage more easily affected by cold, and should it not prove to be the case, it was thought that as a sexual development of the parasite probably ensues within the tick, the cold might retard that sexual development, or else completely inhibit it, in the same way as cold retards the development of the malarial parasite within the mosquito.

At the same time, it was decided to note the influence of cold on larvae of the blue tick, which are the sole survivors of the winter months.

Experiment No. 1.

To infect a beast with East Coast fever for the purpose of collecting engorged nymphae of Rhipicephalus appendiculatus.

Cow 455.—Infested on the 23rd May, 1907, with adults of *Rhipicephalus evertsi*, collected from ox 358, which at the time was suffering from East Coast fever.

Cow 455 died on the 17th June, 1907, from East Coast fever.

Engorged *Rhipicephalus appendiculatus* nymphae were collected from cow 455 three days before death (14th June, 1907).

Experiment No. 2.

To prove that these engorged nymphae of Rhipicephalus appendiculatus *will transmit East Coast fever in their adult stage.*

The nymphae of *Rhipicephalus appendiculatus* which were collected on the 14th June, hatched on the 16th July, thirty-two days after they were collected.

Ox 467.—Infested on the 22nd October, 1907, with adults of *Rhipicephalus appendiculatus* from cow 455.

Piroplasma parvum was noted from the 18th to 21st days, on which latter date the beast was killed.

Post-mortem examination showed all the lesions of East Coast fever.

Conclusion.—The adults of *Rhipicephalus appendiculatus* which, as engorged nymphae, were collected from cow 455, were capable of transmitting East Coast fever.

Experiment No. 3.

To note the effect of exposing engorged nymphae of Rhipicephalus appendiculatus *infected with* Piroplasma parvum, *to a temperature of* 0 *C.*

Some of the engorged nymphae of *Rhipicephalus appendiculatus*, which had been collected from cow 455 on the 14th June, were exposed to a temperature of 0 C. for half an hour daily from the 28th June to 19th July, 1907. Twelve days later—31st July—they hatched.

(*a*) *Ox* 471.—Infested on the 23rd September with four of these adult *Rhipicephalus appendiculatus* (fifty-three days old). *Piroplasma parvum* appeared from the 23rd to 33rd days, on which latter date the beast died.

Post-mortem examination revealed all the typical lesions of East Coast fever.

(*b*) *Calf* 441.—Infested on the 22nd October, 1907, with eight *Rhipicephalus appendiculatus* adults of the same brood which had been exposed to a temperature of 0 C. for half an hour daily from the 28th June to 19th July. (At the date of infestation these adults were ninety-five days old.)

Piroplasma parvum appeared from the 10th to 19th days, and the beast died on the 11th November.

Post-mortem examination revealed all the typical lesions of East Coast fever.

Conclusions.—(1) Engorged nymphae of *Rhipicephalus appendiculatus*, when exposed to a temperature of 0 C., do not hatch as quickly as under normal conditions.

Of the engorged nymphae collected on the 14th June, those exposed to a temperature of 0 C. for half an hour daily from the 28th June to 19th July —twenty-one days—hatched on the 31st July, or forty-six days after collection, whilst those kept in the ordinary Petri dishes at a normal temperature hatched on the 16th July, or thirty-two days after collection.

(2) Engorged nymphae of *Rhipicephalus appendiculatus*, collected from a beast suffering from East Coast fever, and exposed to a temperature of 0 C. for half an hour daily for twenty-one days, were capable of transmitting *Piroplasma parvum* as adults; in one case these adults were fifty-three days old, and in the other ninety-five days old.

Experiment No. 4.

With larvae of Rhipicephalus decoloratus (*the blue tick*).

(*a*) Larval ticks exposed to a temperature of —18 C.

Date.	Time kept at this Temperature.	Result.
July 3, 1908	15 minutes ..	None died.
,, 3, ,, ...	25 ,, ..	Majority died
,, 3, ,, ...	30 ,, ..	All died.
,, 2, ,, ...	60 ,, ..	,,

(*b*) Larval ticks of *Rhipicephalus decoloratus* exposed to a temperature of —5 C.

Date.	Time kept at this Temperature.	Result.
July 5, 1908	5 hours ..	None died.
„ 10, „	10 „ ..	„
„ 11, „	24 „ ..	„
„ 12, „	48 „ ..	Majority died.

Conclusions.—(1) Larval ticks of *Rhipicephalus decoloratus* die when exposed for thirty minutes to a temperature of — 18 C.

(2) Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of — 5 C. for twenty-four hours.

Resume.

1. A temperature of 0 C. retards the hatching of *Rhipicephalus appendiculatus* nymphae into adults.

2. A temperature of 0 C. does not interfere with the development of the parasite within the engorged nymphae.

3. A temperature of 0 C. does not kill the virus contained in engorged nymphae of *Rhipicephalus appendiculatus.*

4. Larval ticks of *Rhipicephalus decoloratus* die within thirty minutes when exposed to a temperature of —18 C.

5. Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of —18 C. for fifteen minutes.

6. Larval ticks of *Rhipicephalus decoloratus* do not die when exposed to a temperature of —5 C. for twenty-four hours.

7. The majority of larval ticks of *Rhipicephalus decoloratus* die when exposed to a temperature of —5 C. for forty-eight hours.

C.—FURTHER EXPERIMENTS WITH BILIARY FEVER IN EQUINES.

Continuing on the lines mentioned in my previous report, numbers of horses and mules were inoculated during the past year against piroplasmosis, and in the majority of cases I utilised donkey foal blood of the fourth, fifth, and sixth generations.

Experiment No. 12—(*continued*).

Fourth Generation.

Argentine horses injected with blood of a Transvaal donkey foal (third generation).

67. *Horse* 2845.—Injected on the 3rd July, 1907, subcutaneously with 3 c.c. blood of donkey foal 2564.
    Typical reaction from the 7th day.
    Piroplasms noted from the 14th day.

68. *Horse* 2840.—Injected on the 3rd July, 1907, subcutaneously with 5 c.c. blood of donkey foal 2564.
    Reaction from the 8th day.
    Piroplasms noted from the 11th day.

69. *Horse* 2975.—Injected on the 15th August, 1907, subcutaneously with 3 c.c. blood of donkey foal 2564.
    Reaction from the 7th day.
    Piroplasms noted from the 10th day.

Results.—Of three Argentine horses injected with blood of a Transvaal donkey foal (third generation) all reacted and recovered.

Experiment No. 13—(*continued*).

Fifth Generation.

Argentine horses injected with blood of Transvaal donkey foal immune against Piroplasma equi.

Horses 2844 and 2846 were injected on the 3rd July, 1907, subcutaneously with blood of donkey foal 2550.

14. *Horse* 2844.—Injected as above. Dose 3 c.c.
Reaction from the 6th day.
Piroplasms noted from the 15th day.
15. *Horse* 2846.—Injected as above. Dose 5 c.c.
Reaction from the 7th day.
Piroplasms noted from the 12th day.

Argentine horses 2962, 2963, and 2964 were injected on the 7th August, 1908, subcutaneously with 3 c.c. defibrinated blood of donkey foal 2550.

16. *Horse* 2962.—Injected as above.
Reaction from the 8th day.
Piroplasms noted from the 11th day.
17. *Horse* 2963.—Injected as above.
Reaction from the 10th day.
Piroplasms noted from the 18th day.
18. *Horse* 2964.—Injected as above.
Reaction from the 6th day.
Piroplasms noted from the 12th day.

Argentine horses 2965, 2966, and 2967 were injected on the 7th August, 1907, subcutaneously with 2 c.c. defibrinated blood of donkey foal 2550.

19. *Horse* 2965.—Injected as above.
Reaction from the 6th day.
Piroplasms noted from the 12th day.
20. *Horse* 2966.—Injected as above.
Reaction from the 9th day.
Piroplasms noted on the 18th and 19th days.
21. *Horse* 2967.—Injected as above.
Reaction from the 9th day.
Piroplasms noted from the 13th day.

Argentine horses 2981, 2982, 2983, and 2984 were injected on the 9th August, 1907, subcutaneously with 2 c.c. blood of Transvaal donkey foal 2550.

22. *Horse* 2981.—Injected as above.
Very slight reaction.
Piroplasms noted on the 13th day.
23. *Horse* 2982.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 12th and 13th days.
24. *Horse* 2983.—Injected as above.
Reaction from the 10th day.
Piroplasms noted on the 12th and 13th days.
25. *Horse* 2984.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 11th, 12th, and 13th days.

Result.—Of twelve Argentine horses injected with blood of a Transvaal donkey foal (fourth generation) all reacted and recovered.

EXPERIMENT No. 14—(*continued*).

Sixth Generation.

Argentine horses injected with blood of Transvaal donkey foal immune against Piroplasma equi.

Horses 2842 and 2843 were injected on the 3rd July, 1907, subcutaneously with blood of Transvaal donkey foal 2551.

4. *Horse* 2842.—Injected as above. Dose 3 c.c.
   Reaction from the 7th day.
   Piroplasms noted from the 12th day.

5. *Horse* 2843.—Injected as above. Dose 5 c.c.
   Reaction from the 8th day.
   Piroplasms noted from the 12th day.

Argentine horses 2968, 2969, and 2970 were injected on the 7th August, 1907, subcutaneously with 3 c.c. blood of Transvaal donkey foal 2551.

6. *Horse* 2968.—Injected as above.
   Reaction from the 9th day.
   Piroplasms noted from the 15th day.

7. *Horse* 2969.—Injected as above.
   Reaction from the 12th day.
   Piroplasms noted from the 13th day.

8. *Horse* 2970.—Injected as above.
   Reaction from the 7th day.
   Piroplasms noted from the 15th day.

Argentine horses 2971, 2972, and 2973 were injected subcutaneously with 2 c.c. defibrinated blood of donkey foal 2551 on the 7th August, 1907.

9. *Horse* 2971.—Injected as above.
   Reaction from the 7th day.
   Piroplasms noted from the 12th day.

10. *Horse* 2972.—Injected as above.
    Reaction from the 9th day.
    Piroplasms noted from the 12th day.

11. *Horse* 2973.—Injected as above.
    Reaction from the 5th day.
    Piroplasms noted on the 14th and 16th days.
    Died from horse-sickness contracted spontaneously on the 6th September, 1907, thirty days after injection with *Piroplasma equi*.

The following Argentine horses were injected on the 9th August, 1907, with 2 c.c. blood of Transvaal donkey foal 2551 :—

12. *Horse* 2985.—Injected as above.
    Reaction from the 10th day.
    Piroplasms present on the 16th and 17th days.

13. *Horse* 2986.—Injected as above.
    Reaction from the 7th day.
    Piroplasms noted on the 11th day.

14. *Horse* 2987.—Injected as above.
    Reaction from the 7th day, recording 105·6 and 106 on the 14th and 18th days respectively.
    Piroplasms noted from the 11th day.

Argentine Horses 2849 and 2841 were injected on the 3rd July, 1907, subcutaneously with blood of Transvaal donkey foal 2494.

15. *Horse* 2849.—Injected as above. Dose 3 c.c.
    Reaction from the 7th day.
    Piroplasms noted on the 15th day.

16. *Horse* 2841.—Injected as above. Dose 5 c.c.
    Reaction from the 7th day.
    Piroplasms noted from the 10th day.

The following Argentine horses were injected on the 7th August, 1907, with blood of Transvaal donkey foal 2494 :—

Dose 3 *c.c.*

17. *Horse* 2974.—Injected as above.
Reaction from the 3rd day.
Piroplasms noted on the 13th, 14th, and 15th days.

18. *Horse* 2976.—Injected as above.
Reaction on the 5th day.
Piroplasms noted on the 12th, 13th, 14th, and 15th days. Secondary reaction from 27th day ; temperature reaching 106·6 on 32nd day, and death occurring on the following day from horse-sickness.

19. *Horse* 2977.—Injected as above.
Reaction from the 7th day.
Piroplasms noted from the 11th day.

Dose 2 *c.c.*

20. *Horse* 2978.—Injected as above.
Reaction from the 10th day.
Piroplasms noted on the 14th day.

21. *Horse* 2979.—Injected as above.
Reaction from the 10th day.
Piroplasms noted on the 15th and 16th days.

22. *Horse* 2980.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 13th, 14th, and 15th days.

The following Argentine horses were injected on the 9th August, 1907, with 2 c.c. blood of Transvaal donkey foal 2494 immune against *Piroplasma equi* (fifth generation) :—

23. *Horse* 2988.—Injected as above.
Reaction from the 7th day.
Piroplasms present on the 11th, 12th, and 13th days.

24. *Horse* 2989.—Injected as above.
Very slight reaction from the 7th day.
Piroplasms noted on the 13th and 14th days.

25. *Horse* 2990.—Injected as above.
Reaction from the 7th day, reaching 105·4 eight days later.
Piroplasms noted from the 13th day.

Results.—Of twenty-two Argentine horses injected with blood of Transvaal donkey foals (fifth generation) all reacted and twenty recovered. The remaining two died of horse-sickness contracted spontaneously.

Experiment No. 15.

Seventh Generation.

Argentine horse injected with blood of Transvaal horse foal 2707 (*sixth generation*) *immune against* Piroplasma equi.

1. *Horse* 2847.—Injected subcutaneously with 3 c.c. blood of Transvaal horse foal on the 3rd July, 1907.
Reaction from the 7th day.
Piroplasms noted from the 8th day.

Argentine mules injected with blood of a Transvaal donkey foal immune against Piroplasma equi.

The following mules were injected on the 25th October, 1907, with 1 c.c. blood of Transvaal donkey foal 2926—immune against *Piroplasma equi* (sixth generation).

(NOTE.—All these mules had previously been immunised and tested against horse-sickness, and were exposed to natural infection at Onderstepoort, near Pretoria, two days after injection with *Piroplasma equi*.)

2. *Mule* 3009.—Injected as above.
   No distinct reaction.
3. *Mule* 3012.—Injected as above.
   Slight reaction.
   Blood examinations negative.
4. *Mule* 3018.—Injected as above.
   Slight reaction from the 7th day.
5. *Mule* 3023.—Injected as above.
   Reaction from the 6th day.
   Blood examinations negative.
6. *Mule* 3025.—Injected as above.
   Very slight reaction.
   The lesions of poikilocytosis noted on the 9th day.
7. *Mule* 3026.—Injected as above.
   Slight reaction.
   Microscopical examinations negative.
8. *Mule* 2893.—Injected as above.
   Very slight reaction.
9. *Mule* 2894.—Injected as above.
   Very slight reaction.
10. *Mule* 2887.—Injected as above.
    Slight reaction from the 8th day.
11. *Mule* 2890.—Injected as above.
    Indistinct reaction.
    Blood examinations negative.
12. *Mule* 2895.—Injected as above.
    Slight reaction from the 8th day.
    Blood examinations negative.
13. *Mule* 2896.—Injected as above.
    Reaction from the 7th day.
14. *Mule* 2892.—Injected as above.
    Reaction from the 6th day.
15. *Mule* 2898.—Injected as above.
    Reaction from the 6th day.
    Blood examinations negative.
16. *Mule* 2897.—Injected as above.
    Reaction from the 6th day.
    Blood examinations negative.
17. *Mule* 2928.—Injected as above.
    No distinct reaction.
18. *Mule* 2889.—Injected as above.
    Slight reaction.
19. *Mule* 2992.—Injected as above.
    Reaction from the 7th day.
    Piroplasms noted five days later.
20. *Mule* 2993.—Injected as above.
    Reaction from the 6th day.
    Piroplasms noted on the 14th day.
21. *Mule* 2994.—Injected as above.
    Slight reaction.
22. *Mule* 2995.—Injected as above.
    No distinct reaction.

23. *Mule* 2996.—Injected as above.
Reaction from the 6th day.
Blood examinations negative.

24. *Mule* 2997.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 17th day.

25. *Mule* 2998.—Injected as above.
Slight reaction.
Piroplasms noted on the 18th day.

26. *Mule* 2999.—Injected as above.
Reaction from the 6th day.
Piroplasms noted five days later.

27. *Mule* 3000.—Injected as above.
Slight reaction.

28. *Mule* 3001.—Injected as above.
Slight reaction.

29. *Mule* 3002.—Injected as above.
Very slight reaction.

30. *Mule* 3003.—Injected as above.
Reaction.

31. *Mule* 2929.—Injected as above.
Slight reaction.

32. *Mule* 2930.—Injected as above.
Slight reaction from the 5th day.

33. *Mule* 2931.—Injected as above.
Slight reaction.

34. *Mule* 2932.—Injected as above.
Slight reaction.

35. *Mule* 2933.—Injected as above.
Slight reaction.

36. *Mule* 2934.—Injected as above.
Slight reaction.

The following Argentine mules were injected on the 11th November, 1907, with 1 c.c. blood of Transvaal donkey foal 2926, sixth generation (these mules had previously been immunised and tested against horse-sickness):

37. *Mule* 3005.—Injected as above.
Very slight reaction.

38. *Mule* 3007.—Injected as above.
Slight reaction.
Blood examinations negative.

39. *Mule* 3015.—Injected as above.
Slight reaction.
The lesions of poikilocytosis noted on the 8th day.

40. *Mule* 3019.—Injected as above.
Reaction from the 7th day.

41. *Mule* 3011.—Injected as above.
Reaction from the 5th day.

42. *Mule* 3016.—Injected as above.
Reaction from the 5th day.
Blood examinations negative.

43. *Mule* 3020.—Injected as above.
Slight reaction.
Piroplasms present on the 9th day
44. *Mule* 3017.—Injected as above.
Reaction from the 7th day.
Blood examinations negative.
45. *Mule* 3006.—Injected as above.
Slight reaction.
Blood examinations negative.
46. *Mule* 3014.—Injected as above.
Reaction from the 5th day.
Blood examinations negative.
47. *Mule* 3024.—Injected as above.
Slight reaction.
48. *Mule* 3010.—Injected as above.
Slight reaction.
Blood examinations negative.
49. *Mule* 3013.—Injected as above.
Reaction from the 5th day.
Blood examinations negative.
50. *Mule* 3021.—Injected as above.
Slight reaction.
No piroplasms noted.
51. *Mule* 3022.—Injected as above.
Slight reaction.

Transvaal donkey foal injected with blood of Transvaal donkey foal immune against Piroplasma equi.

52. *Donkey foal* 3141.—Injected on the 28th November, 1907, with 5 c.c. blood of donkey foal 2926 (sixth generation).

Reaction from the 3rd day, temperature reaching 104 three days later, when the animal died from enteritis.

The following Argentine mules were injected with 1 c.c. blood of Transvaal donkey foal 2926, immune against *Piroplasma equi* (sixth generation), on the 28th November, 1907 (all mules had previously been inoculated against horse-sickness):—

53. *Mule* 3102.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 10th day.
54. *Mule* 3103.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 10th day.
55. *Mule* 3107.—Injected as above.
Slight reaction.
Piroplasms noted on the 13th day.
56. *Mule* 3112.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 12th day.
57. *Mule* 3113.—Injected as above.
Slight reaction.
Piroplasms noted on the 9th day.
58. *Mule* 3114.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 9th day.

59. *Mule* 3099.—Injected as above.
Slight reaction.
Piroplasms noted on the 7th day.
60. *Mule* 3101.—Injected as above.
Reaction from the 4th day.
No piroplasms noted.
61. *Mule* 3108.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 9th day.
62. *Mule* 3109.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 9th day.
63. *Mule* 3106.—Injected as above.
Reaction from the 6th day.
Poikilocytosis noted on the 12th and 13th days.
64. *Mule* 3138.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 13th day.
65. *Mule* 3110.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 12th day.
66. *Mule* 3111.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 9th day.
67. *Mule* 3137.—Injected as above.
Reaction on the 5th day.
Piroplasms noted on the 9th day.
68. *Mule* 3140.—Injected as above.
Reaction from the 4th day.
Piroplasms noted on the 9th day.

Injections on the 28th December, 1907.

69. *Mule* 3179.—Injected as above.
Reaction from the 5th day.
Piroplasms noted from the 9th day.
70. *Mule* 3180.—Injected as above.
Slight reaction.
No piroplasms noted.
71. *Mule* 3181.—Injected as above.
Slight reaction.
No piroplasms noted.
72. *Mule* 3182.—Injected as above.
Slight reaction from the 7th day.
No piroplasms noted.
73. *Mule* 3183.—Injected as above.
Reaction from the 5th day.
No piroplasms noted.
74. *Mule* 3184.—Injected as above.
Slight reaction.
No piroplasms noted.
75. *Mule* 3185.—Injected as above.
Slight reaction.
Piroplasms noted on the 8th day.

76. *Mule* 3186.—Injected as above.
Slight reaction.
No piroplasms noted.
77. *Mule* 3187.—Injected as above.
Slight reaction.
No piroplasms noted.
78. *Mule* 3188.—Injected as above.
Reaction from the 8th day.
No piroplasms noted.

Transvaal horses injected on the 14th January, 1908, with 1 c.c. blood of Transvaal donkey foal 2926 (sixth generation) immune against Piroplasma equi.

79. *Horse* 3119.—Injected as above.
No distinct reaction.
No piroplasms noted.
80. *Horse* 3121.—Injected as above.
Slight reaction.
81. *Horse* 3074.—Injected as above.
Reaction from the 6th day.
82. *Horse* 2904.—Injected as above.
Slight reaction.
83. *Horse* 3130.—Injected as above.
Slight reaction.
84. *Horse* 3065.—Injected as above.
No distinct reaction.
No piroplasms noted.
85. *Horse* 3049.—Injected as above.
No distinct reaction.

Argentine horses injected with 1 c.c. blood of a Transvaal donkey foal on the 17th January, 1908.

86. *Horse* 3248.—Injected as above.
Slight reaction.
Piroplasms noted on the 13th and 14th days.
87. *Horse* 3249.—Injected as above.
Reaction from the 10th day.
Piroplasms noted from the 13th day.
88. *Horse* 3253.—Injected as above.
Reaction from the 8th day.
Piroplasms noted from the 12th day.
89. *Horse* 3254.—Injected as above.
Reaction from the 11th day.
Piroplasms noted from the 12th day.
90. *Horse* 3255.—Injected as above.
Reaction from the 8th day.
Piroplasms noted from the 13th day.
91. *Horse* 3257.—Injected as above.
Slight reaction.
Piroplasms noted from the 13th day.
92. *Horse* 3260.—Injected as above.
Reaction from the 10th day.
Piroplasms noted from the 13th day.
93. *Horse* 3262.—Injected as above.
Slight reaction from the 11th day.
No piroplasms noted.

Injections on the 31st January, 1908.

94. *Horse* 3239.—Injected as above.
No distinct reaction.
No piroplasms noted.

95. *Horse* 3243.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 12th day.

96. *Horse* 3244.—Injected as above.
Reaction from the 6th day.
Piroplasms noted from the 12th day.

97. *Horse* 3246.—Injected as above.
Reaction from the 4th day.
No piroplasms noted.

98. *Horse* 3247.—Injected as above.
Reaction from the 7th day.
Piroplasms noted on the 14th day.

99. *Horse* 3250.—Injected as above.
No distinct reaction.
No piroplasms noted.

100. *Horse* 3252.—Injected as above.
No distinct reaction.
No piroplasms noted.

101. *Horse* 3256.—Injected as above.
Reaction from the 7th day.
No piroplasms noted.

102. *Horse* 3258.—Injected as above.
Reaction from the 6th day.
No piroplasms noted.

103. *Horse* 3259.—Injected as above.
Slight reaction.
Piroplasms noted on the 14th day.

104. *Horse* 3261.—Injected as above.
Reaction from the 3rd day.
Piroplasms noted on the 11th day.

105. *Horse* 3264.—Injected as above.
Slight reaction.
Piroplasms noted on the 14th day.

106. *Horse* 3265.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 13th day.

107. *Horse* 3266.—Injected as above.
Slight reaction.
Piroplasms noted on the 14th day.

108. *Horse* 3267.—Injected as above.
Slight reaction.
No piroplasms noted.

109. *Horse* 3268.—Injected as above.
Slight reaction.
No piroplasms noted.

110. *Horse* 3269.—Injected as above.
Reaction from the 5th day.
Piroplasms noted on the 14th day.

111. *Horse* 3273.—Injected as above.
Reaction from the 6th day.
Piroplasms noted on the 14th day.
112. *Horse* 3274.—Injected as above.
No reaction.
No piroplasms noted.

Results of 112 Animals Injected with Blood of Transvaal Donkey Foals (Sixth Generation).

27 Argentine horses reacted and recovered.
76 ,, mules ,, ,,
7 Transvaal horses ,, ,,
1 ,, donkey foal reacted and died of enteritis.

N.B.—In the majority of cases the temperature reactions were of a slight or indistinct character.

One Transvaal horse foal injected with blood of a Transvaal horse (sixth generation) passed through a reaction and recovered.

Analysis of Results.

1. Of sixty-four Argentine horses injected with blood of Transvaal donkey foals (third, fourth, fifth, and sixth generations) all reacted to piroplasmosis and recovered, except two animals which died of horse-sickness contracted spontaneously.

2. Seventy-six Argentine mules injected with donkey foal blood of the sixth generation reacted and recovered.

3. Seven Transvaal horses injected with donkey foal blood of the sixth generation reacted and recovered.

4. One Transvaal donkey foal injected with donkey foal blood of the sixth generation died of enteritis.

5. One Transvaal horse foal injected with blood of a Transvaal horse reacted and recovered.

The total inoculations obtained by adding these results to those of last year from the injection of donkey foal blood is as follows :—

Seven Transvaal horses injected with donkey foal blood (sixth generation) recovered.

Of three Transvaal horse foals injected with donkey foal blood (second and fifth generations), one died of horse-sickness contracted spontaneously.

One Transvaal mule injected with blood of Transvaal donkey foal (second generation) recovered.

Of five Transvaal donkey foals injected with blood of a Transvaal donkey foal (third, fourth, and sixth generations), one died of enteritis.

Of seventy Argentine horses injected with blood of Transvaal donkey foals (second to sixth generations), two died of horse-sickness contracted spontaneously.

Of eighty Argentine mules injected with blood of Transvaal donkey foals (fourth to sixth generations), none died.

Of ten Argentine donkeys injected with blood of Transvaal donkey foals (second and fourth generations), none died.

Conclusions.

Argentine and Transvaal horses and mules can safely be inoculated against piroplasmosis by using donkey foal blood of the fourth generation and upwards.

D.—THE INOCULATION OF MULES WITH POLYVALENT VIRUS—(*continued*).

The experience of the year 1905–06, when a monovalent virus of the Ordinary strain was utilised for the inoculation of mules in practice, resulted in breakdowns in immunity to the extent of 0·6 per cent. After it had been found that the Tzaneen virus broke the immunity obtained from the Ordinary virus, and it was naturally thought that the virus Tzaneen would protect the animals better in practice, this virus was introduced into practice, with the result that 1·5 per cent. of these animals died after discharge. At the same time it was found that the immunity obtained by either of the above vira could be broken reciprocally and also by a number of other vira which in the meantime we obtained in practice from mules suffering from relapses. Then the idea occurred to unite all these various vira and to obtain in this way a polyvalent virus which would protect against any of the vira of which it was composed, and by this means I hoped to reduce the mortality in practice. In my last annual report I quoted a number of experiments carried out with a trevalent and octovalent mixture of vira, these experiments being conducted with either adequate or inadequate serum. I also showed last year that by immunising with a polyvalent virus and serum a protection was obtained against any of the constituents, which prevented any deaths from the tests, although reactions occurred, and these experiments induced me to make the polyvalency of the virus even stronger by including all the vira which had been used up to that time.

The virus was introduced, and is referred to as CD (composite district); it consists of Ordinary, Tzaneen, Bulawayo, and mixtures of these three, plus a horse which had previously been injected with all three vira, together with relapse and spontaneous cases, the blood being collected in Zoutpansberg, Waterberg, Middelburg, Lydenburg, Rustenburg, Natal, Rhodesia, Lourenco Marques, Onderstepoort, Pretoria, Potchefstroom, and the high veld; all these various vira were injected into one horse (2884), and the resulting virus is called CD.

(*Experiments Nos.* 1 *to* 9, *see Annual Report*, 1906-07, *pages* 192–213.)

EXPERIMENT No. 10.

With polyvalent serum and virus CD.

Serum: Polyvalent.

Inadequate to virus CD 2884.

Injection of 300 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 9th August, 1907.

"A." *Mule* 2929.—Injected as above.

Result.—No reaction.

Tested on the 5th September, 1907, by intrajugular injection of 5 c.c. virus 2884.

Result.—No reaction.

Retested on the 19th September, 1907, by intrajugular injection of 5 c.c. virus 2769 (high veld).

Result.—Reaction from 4th to 11th days.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2808 (Rustenburg II).

Result.—Reaction from 7th to 19th days.

" B." *Mule* 2930.—Injected as above.

Result.—Reaction from 4th day, but not typical for horse-sickness.

Tested on the 5th September, 1907, by intrajugular injection of 5 c.c. virus 2884.

Result.—No reaction.

Retested on the 19th September, 1907, by intrajugular injection of 5 c.c. virus 2770 (Zoutpansberg and Waterberg).

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2785 (Natal, Rhodesia, and Lourenco Marques).

Result.—Reaction from 4th to 9th days.

" C." *Mule* 2931.—Injected as above.

Result.—Mild reaction from 6th to 14th days.

Tested on the 5th September, 1907, by intrajugular injection of 5 c.c. virus 2884.

Result.—No reaction.

Retested on the 19th September, 1907, by intrajugular injection of 5 c.c. virus 2773 (Pretoria and Potchefstroom).

Result.—Slight reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2780 (Middelburg and Lydenburg).

Result.—Reaction and dikkop on 12th day.

" D." *Mule* 2932.—Injected as above.

Result.—Slight reaction from 6th to 16th days.

Tested on the 5th September, 1907, by intrajugular injection of 5 c.c. virus 2884.

Result.—No reaction.

Retested on the 19th September, 1907, by intrajugular injection of 5 c.c. virus 2780 (Middelburg and Lydenburg).

Result.—Slight reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2773 (Pretoria and Potchefstroom).

Result.—No reaction.

" E." *Mule* 2933.—Injected as above.

Result.—Irregular reaction from 7th to 19th days.

Tested on the 5th September, 1907, by intrajugular injection of 5 c.c. virus 2884.

Result.—No reaction.

Retested on the 19th September, 1907, by intrajugular injection of 5 c.c. virus 2785 (Natal, Rhodesia, and Lourenco Marques).

Result.—Reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2770 (Zoutpansberg and Waterberg).

Result.—No reaction.

" F." *Mule* 2934.—Injected as above.

Result.—Doubtful reaction.

Tested on the 5th September, 1907, by intrajugular injection of 5 c.c. virus 2884.

Result.—No reaction.

Retested on the 19th September, 1907, by intrajugular injection of 5 c.c. virus 2808 (Rustenburg II).

Result.—Reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2769 (high veld).

Result.—Reaction.

Results.—Of six mules inoculated with polyvalent serum inadequate to virus CD, one gave no reaction, two gave doubtful reactions, and three gave slight reactions. When tested with constituents of virus CD reactions were again noted.

Experiment No. 11.

With polyvalent serum of horses and mules and virus CD.

Serum : Mixture of 1906 of horses and mules hyperimmunised with various strains.

Inadequate to virus 2884, CD (third generation).

Injection of 300 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 15th August, 1907.

" A." *Mule* 2992.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 5 c.c. virus 2952, CD (fourth generation).

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2769 (high veld).

Result.—Reaction from 4th to 9th days, but not quite typical for horse-sickness.

" B." *Mule* 2993.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 5 c.c. virus 2952, CD (fourth generation).

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2770 (Zoutpansberg and Waterberg).

Result.—No reaction.

" C." *Mule* 2994.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 5 c.c. virus 2952, CD.

Result.—No reaction.

Retested on the 4th October by intrajugular injection of 5 c.c. virus 2773 (Pretoria and Potchefstroom).

Result.—No reaction.

" D." *Mule* 2995.—Injected as above.

Result.—Reaction.

Tested on the 12th September, 1907, by intrajugular injection of 5 c.c. virus 2952, CD

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2780 (Middelburg and Lydenburg).

Result.—Reaction from 4th to 9th days.

" E." *Mule* 2996.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 5 c.c. virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2785 (Natal, Rhodesia, and Lourenco Marques).

Result.—Reaction from 4th to 10th days.

"F." *Mule* 2997.—Injected as above.

Result.—Reaction.

Tested on the 12th September, 1907, by intrajugular injection of 5 c.c virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2808 (Rustenburg II).

Result.—Reaction from 3rd to 8th days.

Results.—Of six mules injected with polyvalent serum of horses and mules inadequate to virus 2884 CD, four gave slight reactions and two strong reactions. When tested later with constituents of virus 2884 some reactions were again noted.

Experiment No. 12.

With polyvalent serum of horses and virus CD.

Serum: Polyvalent mixture of horses.

Inadequate to virus 2884 CD.

Injection of 300 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 15th August, 1907.

"A." *Mule* 2998.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2804 (Rustenburg I).

Result.—Reaction from 7th to 15th days with slight dikkop on 15th day.

"B." *Mule* 2999.—Injected as above.

Result.—Reaction.

Tested on the 12th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—Reaction from 2nd to 14th days, but not typical for horse-sickness.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2809 (Potchefstroom I).

Result.—Reaction from 4th to 10th days.

"C." *Mule* 3000.—Injected as above.

Result.—Reaction.

Tested on the 12th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2810 (Potchefstroom II).

Result.—Reaction from 3rd to 10th days.

"D." *Mule* 3001.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2790 (Onderstepoort).

Result.—Reaction from 4th to 10th days.

"E." *Mule* 3002.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2870 (District).

Result.—No reaction.

"F." *Mule* 3003.—Injected as above.

Result.—Slight reaction.

Tested on the 12th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2599 (spontaneous cases).

Result.—No reaction.

Results.—Of six mules injected with a polyvalent serum mixture of horses inadequate to virus CD, four showed slight reactions and two strong reactions. When tested later with constituents of virus 2884, reactions—and in one case a dikkop—were noted.

Experiment No. 13.

With polyvalent serum in varying doses and virus CD.

Serum: Polyvalent.
Inadequate to virus CD 2884.

(*a*) Injection of 250 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 22nd August, 1907.

"A." *Mule* 3026.—Injected as above.

Result.—Slight reaction.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952 CD.

Result.—Doubtful reaction, not typical for horse-sickness.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2709 Ordinary strain.

Result.—Irregular reaction.

"B." *Mule* 3025.—Injected as above.

Result.—Irregular reaction.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—Reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2870 (District).

Result.—Irregular reaction.

"C." *Mule* 3018.—Injected as above.

Result.—Slight irregular reaction.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—Irregular reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2810 (Potchefstroom II).

Result.—Reaction from 3rd to 8th days.

(*b*) Dose of serum: 200 c.c.

"D." *Mule* 3009.—Injected as above.

Result.—Reaction.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—No reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2804 (Rustenburg I).

Result.—Reaction with a dikkop on the 15th day.

"E." *Mule* 3023.—Injected as above.

Result.—Reaction.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—Doubtful reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2790 (Onderstepoort).

Result.—Reaction from 2nd to 16th days.

"F." *Mule* 3012.—Injected as above.

Result.—Reaction.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—Irregular reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2809 (Potchefstroom I).

Result.—Reaction from 3rd to 14th days.

(*c*) Dose of serum: 150 c.c.

"G." *Mule* 2894.—Injected as above. (Had been previously injected on 27th July, 1907, and gave a doubtful reaction.)

Result.—Slight irregular reaction.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—Slight atypical reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2172 (Bulawayo, ninth generation).

Result.—Reaction.

"H." *Mule* 2893.—Injected as above. (Had been previously injected on 27th July, 1907, and gave a slight reaction.)

Result.—Reaction from 2nd to 16th days.

Tested on the 19th September, 1907, by intrajugular injection of 10 c.c. virus 2952.

Result.—Slight reaction.

Retested on the 4th October, 1907, by intrajugular injection of 5 c.c. virus 2199 (Tzaneen, twelfth generation).

Result.—Reaction from 4th to 10th days.

Results.—Of three mules injected with 250 c.c. polyvalent serum inadequate to virus 2884 C.D., one gave a reaction and two slight reactions. When tested later with constituents of the virus mixture, reactions were again noted.

Of three mules injected with 200 c.c. polyvalent serum inadequate to virus 2884, all gave reactions; when tested later with constituents of the virus CD, reactions were again noticed in every case, and in one instance accompanied with a dikkop.

Of two mules injected with 150 c.c. polyvalent serum inadequate to virus 2884, one gave a doubtful reaction and the other a slight reaction; when tested later with constituents of virus 2884, reactions were again noted.

Experiment No. 14.

With serum and virus CD.

Serum: CD of two horses, dated 28th August, 1907.
Adequate to virus 2884 CD.

Injection of 300 c.c. serum and 2 c.c. virus simultaneously and subcutaneously.

N.B.—Serum CD was obtained from two horses, already hyperimmunised, one with virus OTBLPW, and the other with O, T, and O virus alternately, and finally both hyperimmunised with virus CD.

"A." *Mule* 3005.—Injected as above.
Result.—Slight reaction.
Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus 2884.
Result.—Irregular and doubtful reaction.

"B." *Mule* 3006.—Injected as above.
Result.—Slight reaction.
Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus 2884.
Result.—Irregular doubtful reaction.

"C." *Mule* 3007.—Injected as above.
Result.—Reaction.
Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus 2884.
Result.—Irregular, doubtful reaction.

Results.—Of three mules injected with serum CD, adequate to virus 2884 CD, two gave slight reactions, and one a strong reaction. When tested later with constituents of virus 2884, all three animals gave doubtful reactions.

Experiment No. 15.

With serum and virus CD.

Serum: CD of horses.
Adequate to virus CD 2884.

Injection of 300 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 28th August, 1907.

N.B.—Serum obtained from horses hyperimmunised with CD virus exclusively.

"A." *Mule* 3008.—Injected as above.
Result.—Reaction.
Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus (Total CD).*
Killed on 30th October, 1907, owing to debility.

* Virus "Total CD" is a virus obtained by mixing the constituents of CD.

" B." *Mule* 3010.—Injected as above.

Result.—Slight irregular reaction of a doubtful character.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus (Total CD).

Result.—Slight reaction.

" C." *Mule* 3011.—Injected as above.

Result.—Hardly any reaction.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus (Total CD).

Result.—Slight reaction from 6th to 12th day.

Results.—Of three mules injected with polyvalent serum adequate to virus 2884, one gave a reaction, and two doubtful reactions. When tested later with a mixture of the constituents of virus CD, two slight reactions were noted.

EXPERIMENT No. 16.

With polyvalent serum and virus CD.

Serum: Polyvalent.

Inadequate to virus 2884 CD.

(*a*) Injection of 150 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 5th September, 1907.

" A." *Mule* 3013.—Injected as above.

Result.—Irregular reaction.

Tested on the 24th Oetober, 1907, by subcutaneous injection of 5 c.c. virus (Total CD).

Result.—Irregular reaction.

" B." *Mule* 3014.—Injected as above.

Result.—Reaction.

Tested on the 24th Oetober, 1907, by subeutaneous injection of 5 c.c. virus 2884.

Result.—No reaetion.

" C." *Mule* 3015.—Injected as above.

Result.—Reaction and dikkop.

Tested on the 24th Oetober, 1907, by subeutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

" D." *Mule* 3016.—Injected as above.

Result.—Reaction.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus (Total CD).

Result.—Doubtful reaction.

" E." *Mule* 3021.—Injeeted as above.

Result.—Reaetion and dikkop.

Tested on the 24th October, 1907, by subeutaneous injeetion of 5 e.c. virus (Total CD).

Result.—Doubtful reaetion.

" F." *Mule* 3024.—Injected as above.

Result.—Reaction.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaetion.

Results.—Of six mules injected with 150 c.c. polyvalent serum inadequate to virus 2884, reactions were noted in every case. When tested later no reactions were given against virus CD, but doubtful reactions were noted against a mixture of the constituents of virus CD.

EXPERIMENT No. 17.

With virus and serum CD.

Serum: CD.

Adequate to virus 2884 CD.

Injection of 200 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 19th October, 1907.

"A." *Mule* 3017.—Injected as above.

Result.—Reaction.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

"B." *Mule* 3019.—Injected as above.

Result.—Slight reaction.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

"C." *Mule* 3020.—Injected as above.

Result.—Irregular reaction.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus (Total CD).

Result.—Reaction.

"D." *Mule* 3022.—Injected as above.

Result.—Slight reaction of a doubtful character.

Tested on the 24th October, 1907, by subcutaneous injection of 5 c.c. virus (Total CD).

Result.—No reaction.

Results.—Of four mules injected with 200 c.c. serum CD, adequate to virus CD, reactions and doubtful reactions were given. When tested later, none showed reactions against virus CD, but one out of two gave a reaction against a mixture of the constituents of virus CD.

EXPERIMENT No. 18.

With serum and virus CD.

Serum: CD of four horses, dated 2nd September, 1907.

Adequate to virus CD 2884.

(*a*) Injection of 150 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 10th October, 1907.

"A." *Mule* 3113.—Injected as above.

Result.—Atypical reaction.

Tested on the 14th November, 1907, by subcutaneous injection of 5 c.c. virus 2936 (CD, fourth generation).

Result.—Atypical reaction.

"B." *Mule* 3103.—Injected as above.

Result.—Irregular reaction.

Tested on the 14th November, 1907, by subcutaneous injection of 5 c.c. virus 2936.

Result.—No reaction.

"C." *Mule* 3114.—Injected as above.

Result.—Reaction.

Tested on the 14th November, 1907, by subcutaneous injection of 5 c.c. virus 2936.

Result.—No reaction.

(*b*) Dose of serum 200 c.c.

"D." *Mule* 3112.—Injected as above.

Result.—Irregular reaction.

Tested on the 14th November, 1907, by subcutaneous injection of 5 c.c. virus 2936.

Result.—No reaction.

"E." *Mule* 3102.—Injected as above.

Result.—Reaction.

Tested on the 14th November, 1907, by subcutaneous injection of 5 c.c. virus 2936.

Result.—No reaction.

"F." *Mule* 3107.—Injected as above.

Result.—Reaction.

Tested on the 14th November, 1907, by subcutaneous injection of 5 c.c. virus 2936.

Result.—No reaction.

Results.—Of six mules injected with 150 c.c. and 200 c.c. serum CD, adequate to virus CD, three showed irregular reactions and three strong reactions; when tested later with the next generation of virus CD, five gave negative results and one showed an atypical reaction.

Experiment No. 19.

With virus and serum CD.

Serum: No. 196 and 197 CD of forty-two horses.
Adequate to virus 2884 CD.

(*a*) Injection of 100 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 8th November, 1907.

"A." *Mule* 3138.—Injected as above.

Result.—Reaction.

Exposed from the 11th January, 1908, to 8th March, 1908, at Potgietersrust.

Result.—No reaction.

"B." *Mule* 3139.—Injected as above.

Result.—Contracted horse-sickness and died on the 11th day.

"C." *Mule* 3106.—Injected as above.

Result.—Severe reaction with symptoms of horse-sickness.

Exposed from the 11th January, 1908, to 8th March, 1908, in Potgietersrust.

Result.—No reaction.

(*b*) Dose of serum 150 c.c.

"D." *Mule* 3137.—Injected as above.

Result.—Reaction.

Exposed from the 11th January, 1908, to 8th March, 1908, in Potgietersrust.

Result.—No reaction.

"E." *Mule* 3140.—Injected as above.

Result.—Reaction.

Exposed from the 11th January, 1908, to 8th March, 1908, in Potgietersrust.

Result.—No reaction.

" F." *Mule* 3111.—Injected as above.
Result.—Reaction.
Exposed from the 11th January, 1908, to 8th March, 1908, in Potgietersrust.
Result.—No reaction.

" G." *Mule* 3110.—Injected as above.
Result.—Reaction.
Exposed from the 11th January, 1908, to 8th March, 1908, in Potgietersrust.
Result.—No reaction.

(*c*) Dose of serum 200 c.c.

" H." *Mule* 3109.—Injected as above.
Result.—Reaction.
Exposed from the 11th January, 1908, to 8th March, 1908, in Potgietersrust.
Result.—No reaction.

" I." *Mule* 3108.—Injected as above.
Result.—Reaction.
Exposed on the 11th January, 1908, in Potgietersrust.
Result.—Died of spontaneous horse-sickness on 2nd March, 1908.

" J." *Mule* 3099.—Injected as above.
Result.—Severe reaction. Died on 1st January, 1908, with symptoms of anaemia.

" K." *Mule* 3105.—Injected as above.
Result.—Contracted horse-sickness and died on the 10th day.

" L." *Mule* 3104.—Injected as above.
Result.—Died of horse-sickness on the 12th day.

" M." *Mule* 3101.—Injected as above.
Result.—Reaction.
Exposed from the 11th January, 1908, to 8th March, 1908, in Potgietersrust.
Result.—No reaction.

" N." *Mule* 3100.—Injected as above.
Result.—Reaction. Died on the 17th day from peritonitis and pneumonia necrotica.

Results.—Of three animals injected with 100 c.c. horse serum Nos. 196 and 197, adequate to virus CD, one died of horse-sickness on the 11th day, and two showed reactions. Two months later the surviving animals were exposed in Potgieterust without any relapses occurring.

Of four mules injected with 150 c.c. horse serum Nos. 196 and 197, adequate to virus CD, all showed reaction. Two months later when exposed in Potgietersrust no relapses occurred.

Of seven mules injected with 200 c.c. horse serum Nos. 196 and 197, adequate to virus CD, all gave reactions, and two died of horse-sickness ; when exposed two months later in Potgietersrust one of the mules contracted horse-sickness spontaneously and died.

Experiment No. 20.

With polyvalent serum and virus CD 2884.

(*a*) Serum : Polyvalent No. 198 (rest of sample).
Inadequate to virus 2884 CD.

Injection of 250 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 7th December, 1907.

N.B.—Serum 198=Serum OTB of 1906, consisting of two-thirds horse serum and one-third mule serum of animals hyperimmunised with virus O + T + B.

" A." *Mule* 3185.—Injected as above.

Result.—Slight reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2884.

Result.—Indefinite reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Indefinite reaction.

" B." *Mule* 3179.—Injected as above.

Result.—Doubtful reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2415 (Tzaneen).

Result.—No reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Indefinite reaction.

(*b*) Serum 201 (OTB, as before).

Inadequate to virus 2884 CD.

" C." *Mule* 3187.—Injected as above.

Result.—Slight reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2884.

Result.—Indefinite reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Indefinite reaction.

" D." *Mule* 3183.—Injected as above.

Result—Reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2415.

Result.—Reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Reaction; probably due to test with virus 2415.

(*c*) Serum 202 (OTB, as before).

Inadequate to virus 2884 CD.

" E." *Mule* 3182.—Injected as above.

Result.—Doubtful reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2884.

Result.—No reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

"F." *Mule* 3186.—Injected as above.

Result.—Doubtful reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2415.

Result.—Indefinite reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Indefinite reaction.

(*d*) Serum 203 (OTB, as before).

Inadequate to virus 2884, CD.

"G." *Mule* 3188.—Injected as above.

Result.—Slight reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2884.

Result.—Indefinite reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Indefinite reaction.

"H." *Mule* 3181.—Injected as above.

Result.—Reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2415.

Result.—Indefinite reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Indefinite reaction.

(*e*) Serum 204 (OTB, as before).

Inadequate to virus 2884 CD.

"I." *Mule* 3180.—Injected as above.

Result.—Doubtful reaction.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2884.

Result.—No reaction.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

"J." *Mule* 3184.—Injected as above.

Result.—Reaction and dikkop.

Tested on the 7th January, 1908, by subcutaneous injection of 2 c.c. virus 2415.

Result.—Reaction with dikkop on 14th day.

Retested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

Results.—Of ten mules injected with polyvalent serum, twelve months old, inadequate to virus 2884 CD, three gave strong reactions, accompanied in one case with dikkop; three gave slight reactions and four gave doubtful reactions. Five mules were tested with CD virus, and three gave doubtful reactions and two negative results. The other five mules were tested with virus 2415, with the result that one gave a reaction and dikkop, one a reaction, two doubtful reactions, and one negative.

All ten mules were retested with virus 2884, resulting in one reaction, six doubtful reactions, and three negative.

EXPERIMENT No. 21.

With serum and virus CD.

(*a*) Serum 188, dated 23rd September, 1907.

Adequate to virus 2884 CD.

Injection of 250 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 23rd December, 1907.

N.B.—Serum 188 was obtained from the fifteen horses which were the constituents of serum 197.

"A." *Mule* 3223.—Injected as above.

Result.—Irregular reaction.

Tested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Reaction.

"B." *Mule* 3224.—Injected as above, but virus 2884 given intrajugularly.

Result.—Reaction.

Tested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—Irregular reaction.

(*b*) Serum 192, dated 7th October, 1907.

Adequate to virus CD 2884.

Injection of 250 c.c. serum and 2 c.c. virus simultaneously and subcutaneously.

N.B.—Serum 192 was obtained from seventeen horses, the constituents of serum 197.

"C." *Mule* 3221.—Injected as above.

Result.—Slight reaction.

Tested on the 5th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

"D." *Mule* 3222.—Injected as above, but virus given intrajugularly.

Result.—Reaction and dikkop on 10th day. Died of horse-sickness on 14th day.

(*c*) Serum 197.

Adequate to virus CD 2884.

Injection of 200 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 23rd December, 1907.

"E." *Mule* 3217.—Injected as above.

Result.—Very severe reaction with dikkop.

Tested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

"F." *Mule* 3218.—Injected as above, but dose of serum 250 c.c.

Result.—Reaction.

Tested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

"G." *Mule* 3219.—Injected as above. (Dose of serum, 200 c.c.)

Result.—Severe reaction.

Tested on the 15th January, 1909, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

" H." *Mule* 3220.—Injected as above, but virus intrajugularly.
Result.—Doubtful reaction.
Tested on the 15th January, 1908, by injection of 5 c.c. virus 2884.
Result.—Doubtful reaction.

(*d*) Serum 215 A, dated 21st December, 1907.

Adequate to virus 2884 CD.

Injection of 200 and 250 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 23rd December, 1907.

N.B.—Serum 215 A obtained from twenty-two horses, constituents of serum 197.

" I." *Mule* 3213.—Injected as above.
Result.—Severe reaction.
Tested on the 15th January, 1907, by injection of 5 c.c. virus 2884.
Result.—No reaction.

" J." *Mule* 3214.—Injected as above, but dose of serum 250 c.c.
Result.—Severe reaction with dikkop.
Not tested.

" K." *Mule* 3215.—Injected as above.
Result.—Died of horse-sickness on 11th day.

" L." *Mule* 3216.—Injected as above, but virus intrajugularly.
Result.—Severe reaction with piroplasmosis.
Tested on the 15th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.
Result.—No reaction.

Results.—Of twelve mules injected with serum CD, adequate to virus CD, two gave reactions and dikkop, five strong reactions, two slight reactions, one doubtful, and two died of horse-sickness. When tested later with the same virus—one strong, one slight, and one doubtful—reactions were noted, and six gave negative results; the remaining mule was not tested.

Experiment No. 22.

With serum and virus CD.

(*A*) Serum 218.

Adequate to virus 2884.

Injection of 300 and 250 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 10th January, 1908.

N.B.—Serum 218, obtained from eleven horses, constituents of serum 197.

(*a*) Dose, 300 c.c. serum.

" A." *Mule* 3296.—Injected as above.
Result.—Reaction from 8th to 16th days.

(*b*) Dose of serum, 250 c.c.

" B." *Mule* 3291.—Injected as above.
Result.—Reaction from 10th day, and death of horse-sickness on 14th day.

(*B*) Serum 219.

Adequate to virus 2884 CD.

Injection of 300 and 250 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 10th January, 1908.

N.B.—Serum 219, obtained from twelve horses, constituents of serum 197.

(*a*) Dose of serum, 300 c.c.

" C." *Mule* 3297.—Injected as above.

Result.—Hardly any reaction.

Tested on the 24th January, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

(*b*) Dose of serum, 250 c.c.

" D." *Mule* 3292.—Injected as above.

Result.—Reaction from 7th to 17th days.

Results.—Of four mules injected with serum CD, adequate to virus 2884 CD, one died of horse-sickness, three gave strong reactions, and one a very slight reaction. The latter animal was tested with 5 c.c. same virus, but did not give a reaction.

Experiment No. 22.

With serum OTB and virus CD.

Serum 201 OTB

Inadequate to virus 2884 CD.

Injection of 300 and 350 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 7th February, 1908, followed by a second injection of 200 or 100 c.c. serum seven days later.

(*a*) Dose of serum 300 c.c. and second injection of 100 c.c.

" A." *Mule* 3346.—Injected as above.

Result.—Indefinite reaction.

Tested on the 24th February, 1908, by subcutaneous injection of 2 c.c. virus 3330 (Ordinary, seventy-first generation).

Result.—No reaction.

" B." *Mule* 3347.—Injected as above.

Result.—Reaction.

Tested on the 24th February, 1908, by subcutaneous injection of 2 c.c. virus 3330.

Result.—No reaction.

" C." *Mule* 3349.—Injected as above.

Result.—Incubation time of five days; reaction for seven days; dikkop on 11th day, and death on following night.

" D." *Mule* 3366.—Injected as above.

Result.—Reaction.

Tested on the 21st February, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

" E." *Mule* 3367.—Injected as above.

Result.—Reaction.

Tested on the 21st February, 1908, by subcutaneous injection of 5 c.c. virus 2884.

Result.—No reaction.

(*b*) Dose of serum 350 c.c. followed by second injection of 200 c.c.

" F." *Mule* 3348.—Injected as above.

Result.—Reaction.

Tested on the 24th February, 1908, by subcutaneous injection of 2 c.c. virus 3330.

Result.—No reaction.

(*c*) Dose of serum 300 c.c., followed by second injection of 200 c.c.

" G." *Mule* 3350.—Injected as above.

Result.—Reaction.

Tested on the 24th February, 1908, by subcutaneous injection of 2 c.c. virus 3330.

Result.—No reaction.

Results.—Of seven mules injected with OTB serum, inadequate to virus 2884, and which received a second injection of the same serum on the 7th day, one died of horse-sickness, five showed strong reactions, and one gave a doubtful reaction. When tested later with either a constituent or the virus CD 2884, no reactions were noted.

Experiment No. 23.

With serum mixture 230 *and virus CD.*

Serum 230.

Inadequate to virus 2884 CD.

Injection of 300 c.c. serum and 2 c.c. virus simultaneously and subcutaneously on the 10th March, 1908.

N.B.—Serum mixture 230 consisted of serum known as Dikkop, Tzaneen, Spontaneous cases, and of mules Tzaneen and Bulawayo, together with serum CD.

" A." *Mule* 3418.—Injected as above.

Result.—Incubation three days; died from horse-sickness on the 11th day.

" B." *Mule* 3419.—Injected as above.

Result.—Incubation time three days; dikkop on 12th day, and death from horse-sickness during night of 13th–14th day.

" C." *Mule* 3420.—Injected as above.

Result.—Slight reaction.

Tested on the 15th April, 1908, by subcutaneous injection of 2 c.c. virus 3275 (Simpson).

Result.—No reaction.

Retested on the 7th May, 1908, by subcutaneous injection of 2 c.c. virus 3494 (Potgietersrust).

Result.—No reaction.

" D." *Mule* 3421.—Injected as above.

Result.—Doubtful reaction.

Tested on the 15th April, 1908, by subcutaneous injection of 2 c.c. virus 3287 (Simpson).

Result.—No reaction.

Retested on the 7th May, 1908, by subcutaneous injection of 2 c.c. virus 3494 (Potgietersrust).

Result.—No reaction.

" E." *Mule* 3422.—Injected as above.

Result.—Reaction.

Tested on the 15th April, 1908, by subcutaneous injection of 2 c.c. virus 3308 (Simpson).

Result.—No reaction.

Retested on the 7th May, 1908, by subcutaneous injection of 2 c.c. virus 3494 (Potgietersrust).

Result.—No reaction.

" F." *Mule* 3423.—Injected as above.

Result.—Reaction.

Tested on the 15th April, 1908, by subcutaneous injection of 2 c.c. virus 3415 (Simpson).

Result.—No reaction.

Retested on the 7th May, 1908, by subcutaneous injection of 2 c.c. virus 3494 (Potgietersrust).

Result.—No reaction.

Conclusions.—Of six mules injected with polyvalent serum inadequate to virus 2884, two died of horse-sickness, three gave reactions, and one a doubtful reaction.

When tested later with a constituent of virus 2884, no reactions occurred.

Tabulated Résumé.

Mule No.	Immunisation. Date.	Immunisation. Polyvalent. Serum.	Immunisation. Virus. No.	Immunisation. Virus. Orig.	Immunisation. Virus. Gen.	Immunisation. Result.	First Test. Date.	First Test. Virus. No.	First Test. Virus. Orig.	First Test. Virus. Gen.	First Test. Result.
	1907.						1907.				
2929	Aug. 9	Inadeq.	2884	CD	3	—	Sept. 5	2884	CD	3	—
2930	,,	,,	,,	,,	,,	?	,,	,,	,,	,,	—
2931	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
2932	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
2933	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
2934	,,	,,	,,	,,	,,	?	,,	,,	,,	,,	—
2992	Aug. 15	,,	,,	,,	,,	r	Sept. 12	2952	,,	4	—
2993	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
2994	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
2995	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—
2996	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
2997	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—
2998	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
2999	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	?
3000	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—
3001	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
3002	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—
3003	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—

?—Doubtful reaction.
r—Slight reaction.
R—Typical reaction.
——No reaction.

TABULATED RÉSUMÉ—(*continued*).

SECOND TEST.					THIRD TEST.				
Date.	Virus.			Result.	Date.	Virus.			Result.
	No.	Orig.	Gen.			No.	Orig.	Gen.	
1907.					1907.				
Sept. 19	2769	H. Veld	—	R	Oct. 4	2808	R. II	—	R
,,	2770	Z. and W.	—	—	,,	2785	N., Rh., L.M.	—	R
,,	2773	P. and P.	—	r	,,	2780	M. and L.	—	R & D
,,	2780	M. and L.	—	r	,,	2773	P. and P.	—	—
,,	2785	N., Rh., L.M.	—	R	,,	2770	Z. and W.	—	—
,,	2808	R. II	—	R	,,	2769	H. Veld	—	R
Oct. 4	2769	H. Veld	—	?					
,,	2770	Z. and W.	—	—					
,,	2773	P. and P.	—	—					
,,	2780	M. and L.	—	R					
,,	2785	N., Rh., L.M.	—	R					
,,	2808	R. II	—	R					
,,	2804	R. I	—	R & D					
,,	2809	P. I	—	R					
,,	2810	P. II	—	R					
,,	2790	O. Poort	—	R					
,,	2870	District	—	—					
,,	2599	Spont.	—	—					

R—Typical horse-sickness reaction.
r—Slight reaction.
R & D—Typical reaction accompanied with dikkop.
——No reaction.

TABULATED RÉSUMÉ—(*continued*).

Mule No.	Immunisation.						First Test.					Second Test.				
	Date.	Polyvalent Serum.	Virus.			Result.	Date.	Virus.			Result.	Date.	Virus.			Result.
			No.	Orig.	Gen.			No.	Orig.	Gen.			No.	Orig.	Gen.	
	1907.						1907.					1907.				
3026	Aug. 22	Inadeq.	2884	CD	3	r	Sept. 19	2952	CD	4	?	Oct. 4	2709	O.	70	r
3025	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	R	,,	2870	District	—	r
3018	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	r	,,	2810	P. II	—	R
3009	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—	,,	2804	R. I	—	R & D
3023	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	?	,,	2790	O. Poort	—	R
3012	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	r	,,	2809	P. I	—	R
2894	,,	,,	,,	,,	,,	?	,,	,,	,,	,,	?	,,	2172	Bul.	9	R
2893	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	r	,,	2199	Tz.	12	R
3005	Aug. 28	Adeq.	2884	CD	3	r	Oct. 24	2884	CD	3	?					
3006	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	?					
3007	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	?					
3008	,,	,,	,,	,,	,,	R	,,	—	Total CD	—	Killed on account of debility.					
3010	,,	,,	,,	,,	,,	?	,,	—	,,	—	r					
3011	,,	,,	,,	,,	,,	?	,,	—	,,	—	r					
3013	Sept. 5	Inadeq.	2884	CD	3	r	Oct. 24	—	,,	—	r					
3014	,,	,,	,,	,,	,,	R	,,	2884	CD	3	—					
3015	,,	,,	,,	,,	,,	R & D	,,	,,	,,	,,	—					
3016	,,	,,	,,	,,	,,	R	,,	—	Total CD	—	?					
3021	,,	,,	,,	,,	,,	R & D	,,	—	,,	—	?					
3024	,,	,,	,,	,,	,,	R	,,	2884	CD	3	—					
3017	Sept. 19	Adeq.	2884	CD	3	R	Oct. 24	,,	,,	,,	—					
3019	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—					
3020	,,	,,	,,	,,	,,	r	,,	—	Total CD	—	R					
3022	,,	,,	,,	,,	,,	?	,,	—	,,	—	—					
3113	Oct. 10	,,	2884	CD	3	?	Nov. 14	2936	CD	4	?					
3103	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—					
3114	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3112	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—					

3102	„ 10	„	„	„	„	R	„	„	„	„	—					
3107	„	„	„	„	„	R	„	„	„	„	—					
							1908.									
3138	Nov. 8	Adeq. 196 & 197	„	„	„	R	Jan. 11	Exposed in Potgieters-rust	—	—	No result.					
3139	„	„	„	„	„	Died of horse-sickness										
3106	„	„	„	„	„	R	„	—	—	—	„	„	„			
3137	„	„	„	„	„	R	„	—	—	—	„	„	„			
3140	„	„	„	„	„	R	„	—	—	—	„	„	„			
3111	„	„	„	„	„	R	„	—	—	—	„	„	„			
3110	„	„	„	„	„	R	„	—	—	—	„	„	„			
3109	„	„	„	„	„	R	„	—	—	—	„	„	„			
3108	„	„	„	„	„	R	„	Exposed in Potgieters-rust	—	—	Died of horse-sickness on March 2, 1908.					
3099	„	„	„	„	„	R	—	—	—	—	Died on Jan. 1, 1908, of anaemia.					
3105	„	„	„	„	„	Died of horse-sickness										
3104	„	„	„	„	„	do.										
3101	„	„	„	„	„	R	Jan. 11	Exposed in Potgieters-rust	—	—	No result.					
3100	„	„	„	„	„	R	—	—	—	—	Died on 17th day of peritonitis and pneumonia					
												1908.				
3185	Dec. 7	Inadeq. OTB	2884	CD	3	r	Jan. 7	2884	CD	3	?	Jan. 15	2884	CD	3	?
3179	„	„	„	„	„	?	„	2415	Tz.	4	—	„	„	„	„	?
3187	„	„	„	„	„	r	„	2884	CD	3	?	„	„	„	„	?
3183	„	„	„	„	„	R	„	2415	Tz.	4	R	„	„	„	„	R
3182	„	„	„	„	„	?	„	2884	CD	3	—	„	„	„	„	—
3186	„	„	„	„	„	?	„	2415	Tz.	4	?	„	„	„	„	?
3188	„	„	„	„	„	r	„	2884	CD	3	?	„	„	„	„	?
3181	„	„	„	„	„	R	„	2415	Tz.	4	?	„	„	„	„	?
3180	„	„	„	„	„	?	„	2884	CD	3	—	„	„	„	„	—
3184	„	„	„	„	„	R & D	„	2415	Tz.	4	R & D	„	„	„	„	—
3223	Dec. 23	Adeq. CD	2884	CD	3	r	Jan. 15	2884	CD	3	R					

TABULATED RÉSUMÉ—(*continued*).

Mule No.	Immunisation.						First Test.					Second Test.				
	Date.	Polyvalent Serum.	Virus.			Result.	Date.	Virus.			Result.	Date.	Virus.			Result.
			No.	Orig.	Gen.			No	Orig.	Gen.			No.	Orig.	Gen.	
	1907.						1908.									
3224	Dec. 23	Adeq. CD	2884	CD	3	R	Jan. 15	2884	CD	3	r					
3221	,,	,,	,,	,,	,,	r	,,	,,	,,	,,	—					
3222	,,	,,	,,	,,	,,	Died of horse-sickness										
3217	,,	,,	,,	,,	,,	R & D	Jan. 15	2884	CD	3	—					
3218	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3219	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3220	,,	,,	,,	,,	,,	?	,,	,,	,,	,,	?					
3213	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3214	,,	,,	,,	,,	,,	R & D	—	—	—	—	Not tested.					
3215	,,	,,	,,	,,	,,	Died of horse-sickness										
3216	,,	,,	,,	,,	,,	R	Jan. 15	2884	CD	3	—					
3296	Jan. 10	,,	2884	CD	3	R	—	—	—	—	Not tested.					
3291	,,	,,	,,	,,	,,	Died of horse-sickness										
3297	,,	,,	,,	,,	,,	r	Jan. 24	2884	CD	3	—					
3292	,,	,,	,,	,,	,,	R	—	—	—	—	Not tested.					
3346	Feb. 7	Inadeq. OTB	2884	CD	3	?	Feb. 24	3330	O	71	—					
3347	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3348	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3349	,,	,,	,,	,,	,,	Died of horse-sickness										

3350	,,	,,	,,	,,	,,	R	Feb. 24	3330	O	71	—					
3366	Feb. 8	,,	,,	,,	,,	R	Feb. 21	2884	CD	3	—					
3367	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3393	Feb. 21	,,	,,	,,	,,	R	March 9	,,	,,	,,	—					
3394	,,	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3395	Feb. 28	,,	,,	,,	,,	R	,,	,,	,,	,,	—					
3418	March 10	Inadeq. Polyv.	,,	,,	,,	Died of horse-sickness										
3419	,,	,,	,,	,,	,,	,,	do.					1908.				
3420	,,	,,	,,	,,	,,	r	April 15	3275	Simpson	--	—	May 7	3494	P.P.R.	—	—
3421	,,	,,	,,	,,	,,	?	,,	3287	,,	2	—	,,	,,	,,	1	—
3422	,,	,,	,,	,,	,,	R	,,	3308	,,	2	—	,,	,,	,,	1	—
3423	,,	,,	,,	,,	,,	R	,,	3415	,,	2	—	,,	,,	,,	1	—

r—Slight reaction.
?—Doubtful reaction.
R—Typical reaction.
R & D—Typical reaction accompanied with dikkop.
——No reaction.

Analysis of Results from Tabulated Résumé.

1. The immunisation of mules was introduced with two kinds of sera—(*a*) inadequate to the virus being composed of various monovalent sera, and also some trevalent and octovalent sera used in previous years for experimental purposes (see Annual Report, 1906–07), and (*b*) adequate to the virus 2884, that is of horses hyperimmunised with this virus.

2. Of 60 mules immunised with polyvalent serum inadequate to virus CD, 3 died of horse-sickness, or 5 per cent., the dose of serum being 300 c.c.

3. Of 46 mules immunised with polyvalent serum adequate to virus CD, 6 died, or 13 per cent.,

1	having received	300 c.c.	serum.
2	,, ,,	250 c.c.	,,
1	,, ,,	200 c.c.	,,
2	,, ,,	100 c.c.	,,

4. Based on these results the dose of serum was fixed at 300 c.c.

5. The immunity obtained by either an inadequate or an adequate serum was tested by subsequent inoculations of the CD virus, with the result that (*a*) of 44 mules immunised with serum inadequate to the virus CD, and tested with the same virus, 5 reacted—12 per cent.; (*b*) of 25 mules immunised with serum adequate to the virus CD, and tested with the same virus, 5 reacted —20 per cent.

6. The total result of 69 animals immunised with CD, and tested with the same virus, is therefore that 10 reacted—15 per cent.

7. Twenty-two animals were immunised with CD, and tested, as follows :—

	Result.			
	Reaction.	Reaction and Dikkop.	Reaction and Died.	No Reaction.
	%	%	%	%
4 were tested with Ordinary virus ..	—	—	—	100
4 ,, ,, ,, Simpson virus ..	—	—	—	100
5 ,, ,, ,, Tzaneen, fourth generation	20	20	—	60
9 Were exposed in Potgietersrust	—	—	11	—

8. Twenty-eight animals, immunised with CD virus and tested for the first time with the same virus, were tested again with constituents of virus 2884, with the following result :—

		Result.		
		Reaction.	Reaction and Dikkop.	No Reaction.
2	With High Veld virus	1	—	1
2	„ Zoutpansberg and Waterberg	—	—	2
2	„ Potgietersrust	1	—	1
2	„ Middelburg and Lydenburg	2	—	—
2	„ Natal, Rhodesia, and Lourenco Marques ..	2	—	—
2	„ Rustenburg (2)	2	—	—
3	„ Rustenburg (1)	1	2	—
2	„ Pretoria (1)	2	—	—
2	„ Pretoria (2)	2	—	—
2	„ Onderstepoort	2	—	—
2	„ Total District virus (all above)	2	—	—
1	„ Virus of Spontaneous cases	—	—	1
1	„ Tzaneen, fourth generation	1	—	—
1	„ Tzaneen, twelfth generation.	1	—	—
1	„ Ordinary virus	1	—	—
1	„ Bulawayo virus	1	—	—
28		21	2	5

The percentage being : Reactions, 75 per cent.
Reactions and dikkop, 7 per cent.
No reactions, 18 per cent.

9. Eight animals immunised with CD virus were tested (1) with CD, (2) with constituents of CD, and (3) as under :—

		Result.
1	With Rustenburg (2)..	Distinct reaction.
1	„ Natal, Rhodesia, and Lourenco Marques	„ „
1	„ Middelburg and Lydenburg	Reaction and dikkop.
1	„ Pretoria and Potchefstroom	No reaction.
1	„ Zoutpansberg and Waterberg	„
1	„ High Veld	Distinct reaction.
1	„ Tzaneen	Slight reaction.
1	„ OTB	No reaction.

The total percentage of reactions = 62 per cent.

10. Of 10 animals which were immunised with CD and tested with CD or Tzaneen for the first time, and secondly with CD, 1 showed a distinct reaction to this second test; [this animal (3183) had shown a reaction to the immunisation, and a reaction to the first test with Tzaneen, and a third reaction when tested with the virus with which it was originally immunised].

11. Four animals immunised with CD, and tested for the first time with a non-constituent (Simpson) did not react, neither did they when tested with a second non-constituent (Potgietersrust).

Résumé.

1. The immunisation of mules with a polyvalent virus and an adequate or inadequate serum resulted in an immunity which was not complete. When tested later with the corresponding virus the immunity was broken to the extent of 14 per cent. reactions.

2. The immunity obtained with an inadequate serum and virus was broken by the same virus to the extent of 12 per cent. reactions.

3. The immunity obtained with an adequate serum and virus was broken by the same virus to the extent of 20 per cent. reactions.

4. The immunity obtained by the CD virus was broken by constituents to the extent of 22 per cent. reactions, and no deaths.

5. The immunity obtained by CD virus was broken by non-constituents to the extent of 7 per cent. deaths.

6. The immunity obtained by two injections of CD virus was broken by constituents of this virus to the extent of 82 per cent. reactions.

7. The immunity obtained by two injections of CD virus and one injection of a constituent was broken by constituents to the extent of 62 per cent. reactions.

Conclusions.

1. Animals immunised with a polyvalent virus and tested with the same virus show reactions when subsequently retested with the identical virus.

2. When the immunity was tested with constituents of this virus, breakdowns occurred to a large extent in the first test, and even in the second test, showing that the virus, considered to be polyvalent, did not contain all the constituents which were originally mixed. The fact remains, however, that none of the tested animals died, thus showing that the immunity resulting from the CD virus protected against any of the constituents.

3. For the purposes of the practice, where such severe tests are hardly encountered, the immunity given by this CD virus should prove sufficient.

E.—THE LOSS OF VIRULENCY OF HORSE-SICKNESS VIRUS IN PRACTICE.

On page 85 of my Annual Report for 1906–07 I quoted an instance where a virus (Tzaneen) which had proved virulent on the station became inert after it was introduced into practice. The virus was recalled and again tested on the station, when the virulency was proved beyond doubt, and since another virus, which had formerly given typical horse-sickness reactions, subsequently failed to do so on the station, a thorough investigation was made.

Experiment No 1.

With virus which became inert in vitro.

Virus 2199 (horse, origin Tzaneen, twelfth generation) had proved to be so virulent in the experiments with serum that it caused reactions in animals previously immunised against the first generation of the same strain.

"A." *Horse* 2148.—Injected intrajugularly on the 5th October, 1906, with 5 c.c. virus 2199, for the purpose of hyperimmunisation. On the 2nd day the temperature rose to 104 F., reaching 105 F. twenty-four hours later, and the animal died in the course of the 4th day. Blood was collected on the 9th October, 1906, and preserved in the usual way.

(*a*) Injections with virus 2148, dated 9th October, 1906.

"B." *Horse* 2805.—Injected intrajugularly on the 22nd June, 1907, with 5 c.c. blood of horse 2148 (257 days old).

No symptoms until the 14th day, when a small indication of a reaction appeared: the temperature, however, did not rise above 103 F. in the evening; the animal died on the 10th July, 1907—eighteen days after injection—but as it was in poor condition, and lesions of horse-sickness were absent, the cause of death is somewhat doubtful.

"C." *Horse* 2865.—Injected subcutaneously on the 12th July, 1907, with 5 c.c. virus 2148 (277 days old).

No reaction up to the 12th day, when the animal was injected intrajugularly with 5 c.c. virus 2199 (dated 5th October, 1906, or 293 days old, the same virus with which 2148 was originally injected). A reaction followed, and the animal died on the 7th day—31st July, 1907—from horse-sickness.

"D." *Horse* 2883.—Injected subcutaneously on the 20th July, 1907, with 5 c.c. blood 2148 (285 days old).

No reaction.

Tested on the 2nd September, 1907, by injection of 2 c.c. virus 2920 [(Tzaneen, thirteenth generation); horse 2920 had been injected with 2199.]

Reaction commenced on the 4th day after injection, and the animal died of horse-sickness on the 7th day—9th September, 1907.

Result.—The blood of horse 2199 produced horse-sickness in horse 2148 in October, 1906. The blood of 2148 injected in June and July, 1907 (257 to 285 days old) did not produce horse-sickness, and the animals thus injected succumbed to a virus of the same strain. This latter virus (2199, Tzaneen, twelfth generation), which produced horse-sickness in 2148, also did so in one of the animals (2865), and the following generation (2920, Tzaneen, thirteenth generation) in the other one (2883).

Conclusion.—It is evident from the above that virus 2148 collected in October, 1906, had lost its virulency by June, 1907.

Experiment No. 2.

To note whether it is possible to destroy the virulency of a virus by adding inert to virulent virus in varying proportions, and keeping the mixture for different lengths of time at the ordinary room temperature.

(*a*) Injections with a mixture, dated 1st August, 1907, of inert and virulent virus mixed in equal proportions and kept for over four weeks.

Note.—This mixture was prepared on the 1st August, 1907, by mixing equal quantities of avirulent virus 2148 and virulent virus 2199 (Tzaneen strain, thirteenth and twelfth generations respectively). The mixture was kept at the ordinary room temperature.

"A." *Mule* 2991.—Injected subcutaneously on the 2nd September, 1907, with 2 c.c. of the above mixture (thirty-two days old).

A temperature elevation noted on the 2nd and 3rd days, otherwise no indication of a reaction.

On the 18th September, 1907—sixteen days after injection—the animal was tested on its immunity by the subcutaneous injection of 2 c.c. virus 2199. Typical horse-sickness reaction from the 5th to 11th days, the temperature reaching 105 F. on the 8th day, and the animal recovered.

"B." *Mule* 3050.—Injected subcutaneously on the 11th September, 1907, with 8 c.c. of the above mixture (forty-one days old). No reaction.

Tested on the 4th October, 1907 (twenty-three days after first injection) by a subcutaneous injection of 2 c.c. virus 2199.

Reaction commenced six days later, lasting until the 16th day, and the animal recovered.

Retested on the 8th February, 1908, by an intrajugular injection of 10 c.c. mixture of virus mule 3319 and horse 3095 (Potgietersrust strain).

No reaction, thereby proving immune.

(*b*) Injections with a mixture, dated 11th September, 1907, of inert and virulent virus mixed in the proportion of 1 : 9, and kept for over four weeks.

NOTE.—This mixture was made on the 11th September, 1907, by adding 5 c.c. inert virus 2148 to 45 c.c. virus 2199. The mixture was kept at the ordinary room temperature.

"C." *Horse* 3117.—Injected subcutaneously on the 12th October, 1907, with 20 c.c. of virus mixture, dated 11th September, 1907 (thirty-one days old).

No reaction up to the 19th day

Tested on the 31st October, 1907, by subcutaneous injection of 2 c.c. virus 2199.

After an incubation time of five days a reaction started, and the animal died on the 8th day after injection—8th November, 1907—from horse-sickness.

(*c*) Injections with virus mixture, dated 23rd October, 1907, of inert and virulent virus mixed in equal quantities and kept for less than four weeks.

NOTE.—This mixture was made on the 23rd October, 1907, by mixing equal quantities of virus 2199 to the former mixture (dated 1st August, 1907), and was kept at the ordinary temperature.

"D." *Horse* 3091.—Injected subcutaneously on the 30th October, 1907, with 10 c.c. of above mixture (seven days old).

After an incubation time of six days a reaction started, lasting until the 13th day ; dikkop present on the 12th day, and the animal recovered. Horse 3091 was utilised later for hyperimmunisation.

"E." *Horse* 3142.—Injected on the 12th November, 1907, subcutaneously with 3 c.c. of above mixture (twenty days old).

Reaction started six days later, and the animal died on the 9th day from horse-sickness.

(*d*) Injections with mixture, dated 30th October, 1907, of inert and virulent virus in the proportion of 1 : 5 and kept for fourteen days.

Note.—This mixture was made on the 30th October, 1907, by adding 5 parts of virulent virus 3051 (Tzaneen, fifteenth generation) to 1 part of the mixture, dated 23rd October, 1907, and keeping it at the ordinary room temperature.

"F." *Horse* 3135.—Injected subcutaneously on the 14th November, 1907, with 2 c.c. of above mixture (fourteen days old).

Reaction commenced after five days' incubation, and the animal died on the 9th day—23rd November, 1907—from horse-sickness.

(*e*) Injections with mixture, dated 27th December, 1907, of inert and virulent virus in the proportion of 1 : 20, and kept for at least nine weeks.

Note.—This mixture was made on the 27th December, 1907, by adding to the rest of the bottle which contained the mixture of 23rd October, 1907 [see Experiment 3 (*a*)], virus 2199 in the proportion of 1 : 20, and keeping it at the ordinary room temperature.

"G." *Horse* 3370.—Injected subcutaneously on the 29th February, 1908, with 2 c.c. of above mixture (sixty-three days old).

No reaction.

Tested on the 20th March, 1908—twenty days after the first injection—by subcutaneous injection of 2 c.c. virus 2884 (composite district).

Reaction commenced after an incubation time of four days, and the animal died on the 7th day—27th March, 1908—from horse-sickness.

"H." *Horse* 3409.—Injected intrajugularly on the 10th March, 1908, with 5 c.c. of above mixture (seventy-three days old).

Reaction noticed from about the 13th day, lasting for nine days, which might be interpreted as a retarded horse-sickness reaction.

On the 18th day after injection—28th March, 1908—the animal was tapped, and the blood injected into horse 3427, but with negative results.

Horse 3409 was tested on the 6th April, 1908, by a subcutaneous injection of 2 c.c. virus 2891 (Tzaneen, fourth generation).

Incubation time of three days; reaction for ten days; dikkop present on the 12th day; death occurred on the 13th day—19th April, 1908.

"I." *Horse* 3465.—Injected intrajugularly on the 30th March, 1908, with 2 c.c. of above mixture (ninety-three days old).

An indication of a very slight reaction noted from the 6th to the 12th days. The animal was bled on the 10th day, and 2 c.c. was injected into horse 3410. No reaction in 3410, the animal dying from horse-sickness in May in an experiment with virus Tzaneen (eighth generation).

"J." *Horse* 3488.—Injected intrajugularly on the 11th April, 1908, with 10 c.c. of above mixture (104 days old).

No reaction.

This animal was tested later with virus Tzaneen, fourth generation [see Experiment No. 6 (*a*)].

Results.—A mixture made by adding an inert virus mixture to virulent virus in equal proportions, and keeping it at a temperature of 24 C. for twenty days, produced horse-sickness in the two injected animals. A mixture made by adding 1 part of a mixture of inert virus to 5 parts of virulent virus, and keeping it at a temperature of 24 C. for fourteen days, produced horse-sickness in the injected horse. A mixture made by adding 1 part of a mixture of inert virus to 20 parts of virulent virus, and keeping it at a temperature of 24 C. for nine to fifteen weeks, did not produce horse-sickness in the four injected horses. In two of these injected horses a slight indication of a reaction

was noted, but blood collected at the time of the highest temperature record did not produce horse-sickness when injected into two other horses.

A mixture of inert and virulent virus in equal quantities, and kept for four to six weeks at a room temperature, did not produce horse-sickness in the injected animals.

A mixture made by adding 1 part of virulent virus to 9 parts of avirulent virus, and keeping it for thirty-one days at the ordinary room temperature, did not produce horse-sickness in the injected animal.

Experiment No. 3.

To note the influence of an inert virus mixture on a virus CD (or composite district, being a mixture of blood collected from all over the Transvaal).

(*a*) Injections with a mixture, dated 12th March, 1908.

Note.—This mixture was made on the 12th March, 1908, by adding 400 c.c. virus 2884 (CD) to 100 c.c. of the mixture, dated 27th December, 1907, and keeping it at the ordinary room temperature.

"A." *Horse* 3374.—Injected subcutaneously on the 20th March, 1908, with 2 c.c. of the above mixture (eight days old).

Incubation time of four days, followed by a reaction, and the animal died on the 8th day—28th March, 1908—from horse-sickness.

"B." *Horse* 3382.—Injected subcutaneously on the 30th March, 1908, with 2 c.c. of above mixture (eighteen days old).

No definite reaction; the animal was in very poor condition, frequently fell down, and had to be lifted up daily.

Death occurred on the 14th day, the cause of death being debility, and it is therefore doubtful whether a horse-sickness complication existed.

"C." *Horse* 3483.—Injected intrajugularly on the 11th April, 1908, with 10 c.c. of above mixture (thirty days old).

Reaction followed, and the animal was bled.

Tested on the 7th May, 1908, with 2 c.c. virus mule 2415 (Tzaneen, fourth generation). Contracted horse-sickness and died.

Result.—A mixture made by adding 1 part of an inert virus mixture to 4 parts of virulent virus CD, and kept at the ordinary room temperature for eight days, produced horse-sickness in the injected animal. When this mixture was kept for thirty days, it produced an atypical reaction, but the animal died when tested later.

Experiment No. 4.

To find the influence of the temperature of an incubator on inert and virulent virus mixtures.

(*a*) Injections with mixture, dated 24th March, 1908.

Note.—This mixture was prepared on the 24th March, 1908, by putting into an Ehrlenmeyer flask about 270 c.c. virus 2884, after cultures of this virus had given negative results, and 10 c.c. of the old mixture, dated 27th December, 1907. One flask was kept in an incubator at a temperature of 37 C., and the other was kept at the ordinary room temperature 24 C.

(1) With above mixture kept in the incubator.

"A." *Horse* 3199.—Injected subcutaneously on the 6th April, 1908, with 2 c.c. of the above mixture (thirteen days old).

No reaction.

The animal was used later and died of horse-sickness [see Experiment No. 6 (*b*)].

(2) With mixture kept at room temperature.

" B." *Horse* 3363.—Injected subcutaneously on the 6th April, 1908, with 2 c.c. of above mixture (thirteen days old).

Reaction commenced after an incubation time of four days, and the animal died on the 8th day—14th April, 1908—from horse-sickness.

(*b*) Injections with mixture, dated 15th April, 1908.

NOTE.—This mixture was prepared on the 15th April, 1908, by adding to the original bottle containing the inert virus mixture, dated 23rd October, 1907, virus 2884 CD, after it had been tested on its purity in the proportion of 1 : 25.

(1) Above mixture kept at incubator temperature.

" C." *Horse* 3510.—Injected intrajugularly on the 7th May, 1908, with 10 c.c. of above mixture (twenty-two days old).

An atypical reaction.

This horse went into another experiment on the 30th June, 1908, when it was injected with 2 c.c. virus 3619 (Tzaneen, twentieth generation), and died of horse-sickness.

" D." *Horse* 3302.—Injected subcutaneously on the 7th May, 1908, with 10 c.c. of above mixture (twenty-two days old).

No reaction.

This horse was utilised for another experiment with virus Tzaneen, seventh, twentieth, and twenty-first generations, and recovered.

" F." *Horse* 3489.—Injected intrajugularly on the 21st May, 1908, with 10 c.c. of the above mixture (thirty-six days old).

Incubation time of three days; death from horse-sickness on the 6th day.

Result.—A mixture made by adding 1 part of an inert virus mixture to 27 parts of virulent virus 2884, and kept at a temperature of 37 C. for thirteen days, failed to produce horse-sickness in the injected animal. A similar mixture kept for the same length of time at the ordinary room temperature produced horse-sickness in the injected animal.

A mixture made by adding 1 part of an inert virus mixture to 25 parts of virulent virus 2884, and kept for twenty-two days, failed to produce horse-sickness in the two injected horses. The same mixture kept for thirty-six days produced horse-sickness on the 6th day after injection.

EXPERIMENT NO. 5.

With inert virus mixtures kept at a temperature of 37 C. to ascertain whether the injection of a large quantity of inert virus mixture would produce immunity.

NOTE.—The vira used for the following injections were mixed on the 12th March, 1908 [*vide* Experiment No. 3 (*a*)], and kept in an incubator (1 part inert virus to 4 parts virulent virus).

(*a*) Injections of 20 c.c. of virus mixture.

" A." *Horse* 3488.—Injected intrajugularly on the 20th April, 1908, with 20 c.c. of virus mixture (thirty-nine days old).

No reaction.

Tested on the 7th May, 1908, with virus 2415 (Tzaneen, fourth generation) and died of horse-sickness.

"B." *Horse* 3428.—Had been previously injected on the 20th March, 1908, with virus mule 3368 (Tzaneen, sixth generation), but this virus did not always produce horse-sickness, and 3428 had shown an atypical reaction.

Injected intrajugularly on the 20th April, 1908, with 20 c.c. of above mixture (thirty-nine days old).

No reaction.

Tested on the 7th May, 1908, with virus 2415 (Tzaneen, fourth generation).

Slight reaction from 9th to 17th days.

When hyperimmunised later with Tzaneen virus, nineteenth generation, 3428 died of horse-sickness.

(*b*) Injections of 50 c.c. virus mixture.

"C." *Horse* 3199.—Injected intrajugularly on the 20th April, 1908, with 50 c.c. of above mixture (thirty-nine days old).

No reaction.

Tested on the 7th May with virus 2415 (Tzaneen, fourth generation): contracted horse-sickness after an incubation time of thirteen days; had a short reaction, and dikkop appeared on the 17th day.

When hyperimmunised later with Tzaneen virus (3606, nineteenth generation) 3199 contracted horse-sickness and died.

"D." *Horse* 3493.—Injected intrajugularly on the 20th April, 1908, with 50 c.c. of above mixture (thirty-nine days old).

No reaction.

Tested on the 7th May, 1908, with virus 2415 (Tzaneen, fourth generation).

Reaction with dikkop on the 14th day.

Again tested on the 19th June, 1908, with virus 3440 (Potgietersrust), and showed a slight reaction.

Hyperimmunised on the 11th July, 1908, with virus 3774 (Potgietersrust) and recovered.

Result.—The intrajugular injection of 20 c.c. of an inert virus mixture, thirty-nine days old, failed to produce horse-sickness in the two injected horses, and both died when tested later.

The intrajugular injection of 50 c.c. of an inert virus, thirty-nine days old, failed to produce horse-sickness in the two injected horses. When tested later, both showed reactions and one died.

Résumé.

The experiments prove that a virus may become inert in practice; this avirulency is due to some foreign matter, inasmuch as inert virus added to virulent sterile virus promptly produces avirulency. It is probable that this avirulency is due to the presence of some germ, but the experiments have not been carried out to the extent necessary to determine the nature of this micro-organism.

Conclusions.

1. The avirulency of a virus takes place a certain time after mixing sterile to inert virus.

2. The avirulency takes place more rapidly when the mixture is kept in the incubator than when it is kept at room temperature.

3. The mixture of virulent and inert virus produces different results in injected animals according to the method of inoculation. The same virus

which proves inert after a subcutaneous injection may be virulent for an intrajugular injection.

4. The intrajugular injection of large doses of inert virus does not produce immunity.

5. It is clear that a certain virus may become inert, and therefore this fact influences the preparation and preservation of virus to be used in practice.

F.—ON THE VARIABILITY OF THE VIRULENCY OF A PARTICULAR STRAIN OF HORSE-SICKNESS VIRUS.

Hitherto it has been the experience that a virus of horse-sickness taken at random and injected into susceptible horses or mules, irrespective of quantity or method, has in every instance resulted in reactions, and in the great majority deaths followed. This is borne out by the fact that the simultaneous injections of mules with two or more vira and serum resulted in every instance with immunity against that particular virus, or at least in all instances which were tested with that particular strain (compare Annual Report, 1906–07). After the introduction of the Tzaneen virus into practice, and after the apparent failure of this virus in Natal, it became necessary to elucidate the cause, and a new feature was evident, namely, the variability of the virus. This formed the subject of an extended investigation, the details of which are given hereunder. The tables explain themselves, and I only need refer to the terms "Type" and "Strain."

All the different horse, mule, and donkey vira utilised for the following injections are of the same origin (that is of the same strain, Tzaneen), but owing to the numbers of animals used, I have divided them into six tables with the object of making the experiment more comprehensible.

Virus Tzaneen 1087 is the origin; virus 1965 is the first generation, and the main injections with this virus appear under type 1965 (Experiment No. 1). From virus of the third generation of this type (Natal virus) a mule, 2415, was injected, and main injections with virus of this sub-origin form a separate type, 2415 (Experiment No. 2). A second branch was formed by this Natal virus, called type 2891 (Experiment No. 3). Type 2415 was again divided, the mule 2539 forming a type (Experiment No. 4). Similarly with type 2694 (Experiment No. 5) and with type 2732 (Experiment No. 6), these also being branches from 2415. The term "strain" is used to distinguish between the different kinds of vira—Ordinary, Tzaneen, Bulawayo, etc.

EXPERIMENT

With Mule Virus, Type 1965,

HORSE 1087 TZANEEN

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1906.						1906.	1906.	(days)
I	June 28	Mule	1965	I. 5	1087	Orig.	—	July 5 to 11	7
II	Aug. 4	,,	1995	,,	1965	1	—	Aug. 10 to 15	6
	,, 4	,,	1996	,,	,,	1	—	,, 8 to 13	4
III	,, 11	,,	2034	,,	1996	2	Aug. 12	,, 17 to 20	6
	Dec. 17	Horse	2479	I. 2	*,,	,,	,, 12	—	—
	,, 17	Mules, Natal (simlt. serum)	—	—	,,	,,	,, 12	—	—
	,, 27	Horse	2084	I. 5	*,,	,,	,, 12	Jan. 2 to 4	6
	1907.							1907.	
	Mar. 12	Mule	2691	I. 2	*,,	,,	,, 12	—	—
	,, 12	,,	2692	I. 10	*,,	,,	,, 12	—	—
IV	,, 20	,,	2697	I. 5	*2034	3	—	—	—
	Jan. 9	,,	2539	,,	2479	3	—	—	—

* This virus was returned from practice.

TYPE

I Generation,
II Generation,
III Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.	1907.	(days)
IV	Mar. 12	Horse	2656	I. 5	2084	3	Jan. 4	March 23 to 29	11
	,, 28	,,	2712	S. 2	,,	,,	,, 4	—	—
	April 16	—	—	I. 2	,,	,,	,, 4	—	—
	,, 2	Mule	2731	S. 2	,,	,,	,, 4	—	—
V	Mar. 30	,,	2672	,,	2656	4	Mar. 26	—	—
	April 16	—	—	I. 2	,,	,,	,, 26	—	—
	May 21	—	—	I. 20	,,	,,	,, 26	—	—
	,, 3	Mule	2758	S. 2	,,	,,	,, 26	May 7 to 15	4
	,, 3	Horse	2685	,,	,,	,,	,, 26	,, 10 to 15	7
	1908.								
	April 6	Mule	3368	,,	,,	,,	,, 26	April 12 to 18	6
	,, 6	Horse	3384	,,	,,	,,	,, 26	,, 11 to 16	5

No. 1.

Tzaneen strain, first generation.

(ORIGIN).

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1906.						
6	13	Recovd.	–	Dec. 28	2477	—	OTBLPW	—	R	
5	11	R †								
5	9	Slight R	–	—	—	—	—	—	—	
3	9	R †								
				1907.						
—	—	R ?	1	April 16	I. 2	2268	Tzn.	1	R & D	
			2	Sept. 2	I. 5	3063	H. Veld	2	R †	
—	—	—		—	—	—	—	—	—	
2	8	Slight R	–	—	—	—	—	—	—	Killed on account of debility, 9/1/07.
—	—	Irreg. R		Mar. 28	S. 2	2415	Tzn.	4	R	Continued.
—	—	,,	1	,, 28	,,	1965	,,	1	Atyp. R	
			2	April 16	,,	2415	,,	4	R	Continued.
—	—	No R	–	,, 4	,,	2629	,,	5	R †	
—	—	Atyp. R	–	Jan. 22	,,	2415	,,	4	R	Continued.

R†—Reaction and died. R?—Doubtful reaction. R & D—Reaction and dikkop. R—Reaction.

1965.

Mule 1965.
Mule 1996.
Horse 2084.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1907.						
6	17	R †								
—	—	No R	–	—	—	—	—	—	—	
—	—	,,	–	May 3	I. 2	2415	Tzn.	4	R D †	Simult. 400 c.c. serum.
—	—	,,	–	April 16	I. 5	Natal	116/97	—	R	Continued.
—	—	,,	–	—	—	—	—	—	—	
—	—	,,	–	—	—	—	—	—	—	
—	—	,,	–	—	—	—	—	—	—	
8	12	Recovd.	1	May 28	S. 2	2415	Tzn.	4	Irreg. R	
					S. 8	2418	—	—	No R	
			2	June 10		2284	—	—		
						2463	—	—		
						2565	—	—		
					S. 6	2709	Ord.	—	,,	
				,, 21		2148	Tzn.	—		
						2168	Bul.	—		
5	12	R & D	–	July 16	T.9000	2876	—	—	,,	
				1908.						
6	12	Recovd.	–	May 7	T. 2	3494	P.P.R.	1	R	
5	10	R †								

R†—Reaction and died. R & D—Reaction and dikkop. RD†—Reaction with dikkop and died. R—Reaction.

EXPERIMENT

TYPE

III Generation,
IV Generation,
V Generation,
V Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1907.	1908.	(days)
VI	Feb. 26	Mule	3293	S. 2	2685	5	May 12	March 4 to 11	7
	„ 26	Horse	3345	„	„	„	„ 12	„ 6 to 10	9
	April 23	„	3396	„	3368	„	1908. April 14	April 28 to May 3	5
	May 21	Mule	3537	„	„	„	„ 14	May 29 to June 4	8
VII	June 4	Donkey foal	3574	I. 10	3537	6	June 1	—	—
	Mar. 20	Horse	3439	S. 2	3293	6	Mar. 9	—	—
	April 11	—	—	I. 2	„	„	„ 9	—	—
	„ 23	Horse	3459	S. 2	„	„	„ 9	—	—

No. 1—(*continued*).

1965.

Horse 2084.
Horse 2656.
Horse 2685.
Mule 3368.

Reaction.	Incub. + R.	Result.		Tested.	Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
7	14	Recovd.	–	April 6	S. 2	3308	Simp.	—	No R	
				May 7	,,	3494	P.P.R.	1	Slight R	
					,,	3501	CD VI	1	,,	
4	13	R †								
5	10	,,								
6*	14	Recovd.	–	June 11	S. 8	2884	CD	3	R	Complicated piroplasmosis.
						3272	CD V	1	,,	
						3501	CD VI	1	,,	
						3494	P.P.R.	1	,,	
—	—	Atyp. R	–	—	—	—	—	—	—	
—	—	No. R.	–	—	—	—	—	—	—	
—	—	,,	–	April 23	S. 2	3248	Tzn.	6	R D †	
—	—	,,	–	May 21	,,	3398	,,	6	,,	Simult. 200 c.c. serum.

R†—Reaction and died. R—Reaction. RD†—Reaction with dikkop and died.

EXPERIMENT

TYPE

I Generation,
II Generation,
III Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1906.						1906.		(days).
IV	Dec. 21	Mule	2415	S. 10	Natal	116/97	Dec. 21	Dec. 28, 1906, to Jan. 3, '07	7
								1907.	
	,, 27	Horse	2113	I. 10	,,	116	,, 21	Jan. 1 to 4	5
	1907. Mar. 12	Mule	2690	,,	,,	116/97	,, 21	—	—
	,, 24	Horse	2713	,,	,,	,,	,, 21	April 2 to 3	9
	April 16	Mule	2731	I. 5	,,	,,	,, 21	,, 22 to 30	6
	June 10	,,	2802	,,	,,	,,	,, 21	—	—
	,, 10	Horse	2812	,,	,,	,,	,, 21	June 23 to 24	13
	July 9	Mule	2891	,,	,,	,,	,, 21	July 18 to 22	9
	1908.						1907.		
V	Feb. 26	,,	3284	S. 2	2731	4	April 25	—	—
	April 20	—	—	I. 10	,,	,,	,, 25	—	—
	,, 20	Horse	3500	S. 2	,,	,,	,, 25	April 28 to May 5	8
	June 5	Mule	3533	,,	,,	,,	,, 25	June 10 to 20	5
	,, 5	Horse	3616	,,	,,	,,	,, 25	,, 12 to 17	7
							1908.		
VI	,, 26	Mule	3700	,,	3533	5	June 16	—	—

No. 1—(*continued*).

1965.

Mule 1965.
Mule 1996.
Mules, Natal.

Reaction.	Incub. + R.	Result.	Tested.		Virus.				Result.	Remarks.
					Inj.	Orig.	Qual.	Gen.		
(days)	(days)			1907.						
6	13	R & D	1	Feb. 5	S. 2	1938	Ord.	—	R	
			2	May 3	S. 6	2709	,,	—	,,	
						2148	Tzn.	—	,,	
						2168	Bul.	—	,,	
3	8	R †								
—	—	No R	1	Mar. 28	S. 2	2415	Tzn.	4	No R	
			2	April 16	I. 2	2629	,,	5	,,	
			3	May 7	S. 2	2480	OTBLPW	—	,,	Simult. 50 c.c. serum.
1	10	R D †								
8	14	Recovd.	1	,, 21	S. 2	2480	OTBLPW	—	No R	Simult. 50 c.c. serum.
			2	June 4	,,	,,	,,	—	Atyp. R	
			3	,, 19	S. 6	2709	Ord.	—	No R	
						2148	Tzn.	—	,,	
						2168	Bul.	—	,,	
—	—	No R	–	July 15	I. 5	2628	Ord.	—	R †	
1	14	R †								
4	13	R †								
				1908.						
—	—	No R	–	—	—	—	—	—	—	
—	—	Indef. R	1	May 7	S. 2	3500	Tzn.	5	No R	
			2	June 2	,,	2884	CD	3	,,	Simult. 100 c.c. serum.
						3272	CD V	1	,,	
						3501	CD VI	1	,,	
						3494	P.P.R.	1	,,	
7	15	R & D	–	May 24	—	—	—	—	—	Died under infusion.
10	15	Recovd.	–	June 26	S. 3	Poly. VI	—	—	Irreg. R	
5	12	R D †								
—	—	No R	1	July 31	S. 2	3699	Tzn.	6	R	
			2	Aug. 24	S. 3	Poly. VI	—	—	No R	24 hours later, 100 c.c. serum.

R†—Reaction and died. R D†—Reaction with dikkop and died. R—Reaction.

Analysis of Results—Type 1965.

Results.

"A." *Susceptibility.*

1. Of 9 subcutaneous injections into susceptible horses, 2 reacted and recovered, 4 reacted and died, 3 did not react.
2. Of 8 intrajugular injections into susceptible horses. 1 reacted and recovered, 4 reacted and died, 3 did not react.
3. Of 9 subcutaneous injections into susceptible mules, 6 reacted and recovered, 3 did not react.
4. Of 13 intrajugular injections into susceptible mules, 5 reacted and recovered, 3 reacted and died, 5 did not react.

	Subcutaneous injections.		Intrajugular injections.	
	Horses.	Mules.	Horses.	Mules.
	%	%	%	%
Reactions	66	64	62	64
Deaths	44	—	50	24
No reactions	34	33	38	36

Conclusions.

1. The susceptibility of horses and mules is about the same.
2. The subcutaneous injection of virus, type 1965, is more fatal for horses than for mules.
3. The intrajugular injection of virus, type 1965, is more fatal for horses than for mules.
4. The intrajugular injection into any animal (horse or mule) is more fatal than the subcutaneous injection.

Note.—The above results do not include animals which did not react to the injection and later proved to be immune.

"B." *Resistance.*

Results.—Two animals which did not react to the first subcutaneous injection, nor to a second intrajugular injection, did not prove to be immune when injected with the same strain, same generation, but of a different type.

Two horses and three mules which resisted the injection of 2 or 5 c.c. virus, either intrajugularly or subcutaneously showed a horse-sickness reaction when tested with the same strain, different type, and about the same generation. Another mule reacted to the test with a different strain.

Results.

"C." *Virulency.*

1. Of 5 susceptible horses injected with horse virus, 1 reacted and recovered, 3 reacted and died, 1 did not react.
2. Of 5 susceptible mules injected with horse virus. 4 reacted and recovered, 1 did not react.
3. Of 10 susceptible horses injected with mule virus, 2 reacted and recovered, 5 reacted and died, 3 did not react.
4. Of 15 susceptible mules injected with mule virus, 5 reacted and recovered, 3 reacted and died, 7 did not react.

Injections into.	Injections with					
	Horse Virus.			Mule Virus.		
	Reactions.	Died.	No Reaction.	Reactions.	Died.	No. Reaction.
Horses	80 %	60 %	20 %	70 %	50 %	30 %
Mules	100 „	20 „	nil	53 „	20 „	47 „

Conclusions.

1. Mule virus was less virulent than horse virus.

2. Horse virus caused the greatest percentage of reactions.

3. The greatest mortality was caused by the injection of horse virus into horses and mule virus into horses.

4. The least mortality was caused by (1) the injection of horse virus into mules, and (2) by mule virus into mules.

"*D.*" *Variability.*

NOTE.—The subjoined and subsequent tables have been compiled with the object of showing (*a*) the genealogy of the vira, and (*b*) the percentage of reactions (R) given by the individual vira, only taking into consideration two or more injections. (M=Mule; H=Horse.)

1st Generation	...	M. 1965.	
2nd Generation	...	M. 1996—40% R. (5 animals).	
3rd Generation	...	H. 2084—25% R. (4 animals).	Natal M.—85% R. (7 animals).
4th Generation	...	H. 2656—100% R. (4 animals).	M. 2731—75% R. (4 animals).
5th Generation	...	H. 2685—100% R. (2 animals).	
6th Generation	...	M. 3293—Nil (3 animals).	

Conclusions.

1. The virulency of the virus varies in the different generations and depends on the injected animals.

2. It varies in the horse from 25 to 100 per cent., and in the mule from nil to 85 per cent.

3. The virus after passing through horses into a mule lost its virulency.

4. The generation had no influence on the virulency of the virus.

"*E.*" *Influence of dose and manner of injection.*

Injections of virus of type 1965.

1. In the dose of 2 c.c. subcutaneously, 8 reacted and recovered, 3 reacted and died, 5 did not react.
   Of these 5 non-reacters, 3 were tested later by subcutaneous injections of 2 c.c., 2 reacted and died, 1 reacted and recovered:
   and 2 were tested intrajugularly with 2 c.c. and 5 c.c. respectively, 1 reacted and died, 1 reacted and recovered.
2. In the dose of 10 c.c. subcutaneously, 1 reacted and recovered.
3. In the dose of 2 c.c. intrajugularly, 2 did not react. When tested later by intrajugular and subcutaneous injection of 2 c.c. respectively, both reacted and recovered.
4. In the dose of 5 c.c. intrajugularly, 4 reacted and recovered, 5 reacted and died, 3 did not react.
   Of these 3 non-reacters, 2 were tested later by subcutaneous injections of 2 c.c., both reacted and died. One was tested with intrajugular injection of 5 c.c. (different strain) and died.
5. In the dose of 10 c.c. intrajugularly, 2 reacted and died, 1 did not react. When tested later by subcutaneous injection of 2 c.c., this animal reacted and recovered.

EXPERIMENT

With Mule Virus, Type 2415,

HORSE 1087, TZANEEN

I Generation.
II Generation.
III Generation.
IV Generation.

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.	1907.	(days)
V	Jan. 22	Mule	2539	S. 2	2415	4	Jan. 2	Jan. 26 to Feb. 2	3
	Feb. 4	Horse	2629	,,	,,	,,	,, 2	Feb. 9 to 17	6
	,, 4	Mule	2597	,,	,,	,,	,, 2	,, 11 to 19	7
	,, 27	,,	2662	,,	,,	,,	,, 2	March 4 to 11	5
							1906.		
	Mar. 20	,,	2669	S. 5	,,	,,	Dec. 30	March 25 to April 1	5
							1907.		
	,, 28	Horse	2715	S. 2	,,	,,	Jan. 2	April 4 to 10	7
	,, 28	Mule	2690	S. 2	,,	,,	,, 2	—	—
	,, 28	,,	2691	,,	,,	,,	,, 2	,, 4 to 10	7
	April 2	,,	2732	,,	,,	,,	,, 2	—	See
	,, 4	,,	2694	,,	,,	,,	,, 2	—	See
	,, 4	,,	2696	,,	,,	,,	,, 2	,, 10 to 16	6
	,, 16	,,	2692	,,	,,	,,	,, 2	,, 22 to 29	6
	,, 23	Horse	2651	,,	,,	,,	,, 2	,, 28 to May 4	5
	June 20	Donkey foal	2834	T. 5	,,	,,	,, 2	June 24 to July 3	4

No. 2.

Tzaneen strain, 4th generation.

(Origin).

Mule 1965.
Mule 1996.
Mules, Natal.
Mule 2415.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1907.						
7	10	Recovd.	1	Feb. 5	S. 2	2407	Ord.	38	Reactn.	
			2	April 16	,,	2406	OTBLPW	—	,,	
7	13	R †								
8	15	Recovd.	1	Feb. 26	S. 2	2598	Ord.	—	R (late)	
			2	April 17	,,	2406	OTBLPW	—	No R	
7	12	,,	1	Mar. 20	,,	1965	Tzn.	1	Atyp. R	
			2	April 4	,,	2268	,,	—	No R	
			3	May 7	S. 6	2709	Ord.	—	,,	
						2148	Tzn.	—		
						2168	Bul.	—		
7	12	,,	4	16 April	S. 2	2406	OTBLPW	—	R	Simult. 150 c.c. scrum.
			2	May 7	S. 6	2709	Ord.	—	No R	
						2148	Tzn.	—		
						2168	Bul.	—		
6	13	R †								
—	—	No R	1	April 16	I. 2	2629	Tzn.	—	,,	
			2	May 7	S. 2	2480	OTBLPW	—	No R	Simult. 50 c.c. serum.
6	13	Recovd.	1	April 16	,,	2406	,,	—	Indef. R	Simult. 150 c.c. serum.
			2	May 7	S. 6	2709	Ord.	—	No R	
						2148	Tzn.	—		
						2168	Bul.	—		
later	—	—	–	—	—	—	—	—	—	
later	—	—	–	—	—	—	—	—	—	
6	12	Recovd.	1	April 23	S. 2	2406	OTBLPW	—	Indef. R	Simult. 100 c.c. serum.
			2	May 14	S. 5	2709	Ord.	—	No R	
						2148	Tzn.	—		
						2168	Bul.	—		
7	13	R & D	–	,, 21	S. 2	2480	OTBLPW	—	,,	Simult. 50 c.c. serum.
6	11	R D †								
9	13	Recovd.	–	—	—	—	—	—	—	

R—Reaction. R†—Reaction and died. RD—Reaction with dikkop.
RD†—Reaction. dikkop. and died.

EXPERIMENT

TYPE

IV Generation,
V Generation,
V Generation,
V Generation,

Gen.	Date of Injection.	Species.	No.	VIRUS.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1907.	1907.	(days)
V	Jan. 28	Mule	3227	S. 2	2415	4	Jan. 2	Jan. 31 to Feb. 4	3
							1906.		
	April 18	Horse	3465	,,	,,	,,	Dec. 29	April 26 to May 2	8
	,, 18	Mule	3278	,,	,,	,,	,, 29	April 24 to 30	6
	,, 20	,,	3279	,,	,,	,,	,, 29	April 25 to May 1	5
	,, 20	,,	3285	,,	,,	,,	,, 29	April 25 to May 2	5
	,, 20	,,	3390	,,	,,	,,	,, 29	April 26 to May 4	6
	,, 20	,,	3225	—	—	—	—	—	—
	May 14	Horse	3484	S. 2	2415	4	Dec. 29	May 19 to 24	5
	1907.						1907.		
VI	April 4	,,	2706	,,	2669	5	Mar. 29	April 11 to 16	7
	,, 2	Mule	2733	,,	2662	5	,, 8	,, 9 to 16	7
	1908.								
	April 20	Horse	3499	,,	,,	,,	,, 8	,, 26 to 30	6
	,, 20	,,	3495	,,	2692	5	April 24	May 2 to 5	12

No. 2—(*continued*).

2415.

Mule 2415.
Mule 2669.
Mule 2662.
Mule 2692.

Reaction.	Incub. + R.	Result.	Tested.		Virus.				Result.	Remarks.
					Inj.	Orig.	Qual.	Gen.		
(days)	(days)			1908.						
4	7	R D †								
6	14	Recovd.	1	Aug. 7	S. 2	3375	Tzn.	18	No R	Simult. 20 c.c. serum.
			2	June 19	„	3513	CD VI	2	R	
			3	July 13	T. 10000	3735	„	2	No R	
6	12	Slight R	–	May 7	S. 2	3494	P.P.R.	1	R	
						3501	CD VI	1		
6	11	Recovd.	–	June 2	S. 8	Poly. CD	—	—	Slight R	Simult. 50 c.c. serum.
7	12	R & D	–	„	„	„	—	—	No R	„ „
8	14	Recovd.	–	„	„	„	—	—	R	„ „
—	—	R †								
5	10	R D †								
5	12	„								
7	14	R †								
4	10	R †								
3	15	R D †								

R—Reaction. R†—Reaction and died. RD—Reaction and dikkop.
RD†—Reaction with dikkop and died.

EXPERIMENT

TYPE

IV Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.	1907.	(days)
V	April 4	Mule	2694	S. 2	2415	4	Jan. 2	April 9 to 18	5
	1908.								
VI	July 15	„	3699	„	2694	5	April 14	July 20 to 31	5
							1908.		
VII	„ 31	Horse	3745	„	3699	6	July 27	Aug. 7 to 13	7
	„ 31	Donkey foal	3659	S. 5	„	„	„ 27	„ 7 to 14	7
	„ 31	„	3660	I. 5	„	„	„ 27	Aug. 7 to 12	7
	„ 31	Mule	3525	S. 2	„	„	„ 27	„ 5 to 11	5
	„ 31	„	3649	„	„	„	„ 27	„ 5 to 15	5
	„ 31	„	3652	„	„	„	„ 27	Aug. 5 to 11	5
	„ 31	„	3653	„	„	„	„ 27	„ 6 to 14	6
	„ 31	„	3700	„	„	„	„ 27	„ 6 to 12	6
VIII	Aug. 14	Horse	3479	S. 3	3659	7	Aug. 11	Aug. 19 to Sept. 1	4
	„ 14	„	3685	S. 3	3660	7	„ 11	—	—

No. 2—(*continued*).

2415.

Mule 2415.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1907.						
9	14	R & D	1	April 23	S. 2	2406	OTBLPW	—	No R	Simult. 100 c.c. serum.
			2	May 14	S. 5	2709	Ord.	—	„	
						2148	Tzn.	—		
						2168	Bul.	—		
				1908.						
11	16	Recovd.	–	Aug. 24	S. 3	Poly. VI	—	—	No R	After 24 hrs. 100 c.c. serum.
6	13	R †								
7	14	Recovd.	–	—	—	—	—	—	—	
5	12	„	–	—	—	—	—	—	—	
6	11	R †								
10	15	Recovd.	–	Aug. 24	S. 3	Poly. VI	—	—	No R	After 24 hrs. 100 c.c. serum.
6	11	R †								
8	14	„	–							
6	12	Recovd.	–	Aug. 24	S. 3	Poly. VI	—	—	No R	After 24 hrs. 100 c.c. serum.
14	18	R D †								
—	—	No R	1	Aug. 22	S. 3	3699	Tzn.	6	R & D	Simult. 300 c.c. serum.
			2	Sept. 9	„	Poly. VI	—	—	No R	„ „
			3	Oct. 14	T. 10000	3934	P.P.R.	—	„	

R & D—Reaction and dikkop. R†—Reaction and died. RD†—Reaction with dikkop and died.

EXPERIMENT

TYPE

IV Generation,
V Generation,
V Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.	1907.	(days)
VI	Mar. 30	Horse	2686	S. 2	2597	5	Feb. 14	—	—
	April 16	—	—	I. 2	,,	,,	,, 14	April 21 to 25	5
	1908.								
	Feb. 26	Mule	3389	S. 2	,,	,,	,, 14	—	—
	April 22	—	—	I. 10	,,	,,	,, 14	—	—
	Mar. 20	Horse	3451	S. 2	,,	,,	,, 14	—	—
	April 20	,,	3448	I. 10	,,	,,	,, 14	April 30 to May 3	10
	June 19	Mule	3567	I. 50	,,	,,	,, 14	June 29 to July 5	10
	1907.								
	Mar. 20	,,	2639	S. 2	2629	5	,, 14	March 28 to April 3	8
	,, 26	,,	2730	,,	,,	,,	,, 14	April 4 to 9	7
	April 16	,,	2690	I. 2	,,	,,	,, 14	—	—
	,, 4	,,	2693	S. 2	,,	,,	,, 14	April 8 to 16	4
	,, 4	,,	2695	,,	,,	,,	,, 14	April 10 to 16	6
	,, 4	,,	2697	,,	,,	,,	,, 14	,, 8 to 13	4
	1908.								
	April 11	Horse	3443	,,	,,	,,	,, 14	,, 15 to 18	4

No. 2—(*continued*).

2415.

Mule 2415.
Mule 2597.
Horse 2629.

Reaction.	Incub. + R.	Result.	Tested.		Virus.				Result.	Remarks.
					Inj.	Orig.	Qual.	Gen.		
(days)	(days)			1908.						
—	—	No R	–	—	—	—	—	—	—	
4	9	R †								
—	—	Indef. R		—	—	—	—	—	—	
—	—	No R	1	June 3	S. 2	3361	Tzn.	7	R	
			2	„ 26	S. 3	Poly. VI	—	—	No R	
—	—	Indef. R	1	April 20	S. 2	3398	Tzn.	6	R	
			2	May 23	T.8500	3544	Simp. III	—	No R	
3	13	R D †								
6	16	R †								
				1907.						
6	14	R & D	1	April 16	S. 2	2406	OTBLPW	—	No R	Simult. 150 c.c. serum.
			2	May 3	„	2418	OTB	—	„	
						2709	Ord.	—		
			3	„ 21	S. 6	2148	Tzn.	—	„	
						2168	Bul.	—		
5	12	R †								
—	—	No R	1	May 7	S. 2	2480	OTBLPW	—	No R	Simult. 50 c.c. serum.
						2709	Ord.	—		
			2	„ 21	S. 6	2148	Tzn.	—	„	
						2168	Bul.	—		
8	12	Recovd.	1	April 23	S. 2	2406	OTBLPW	—	Slight R	Simult. 100 c.c. serum.
			2	May 14		2709	Ord.	—		
					S. 5	2148	Tzn.	—	No R	
						2168	Bul.	—		
6	12	„	1	April 24	S. 2	2406	OTBLPW	—	Slight R	„ „ ..
			2	May 14		2709	Ord.	—		
					S. 5	2148	Tzn.	—	No R	
						2168	Bul.	—		
5	9	R †								
				1908.						
3	7	Slight R	–	April 27	S. 2	2891	Tzn.	4	R †	

R—Reaction. R†—Reaction and died. RD†—Reaction with dikkop and died.

EXPERIMENT

TYPE

IV Generation.
V Generation,

Gen.	Date of Injection.	Species.	No.	VIRUS.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1907.	1908.	(days)
VI	Aug. 14	Mule	3788	S. 2	2694	5	April 11	Aug. 18 to 29	3
	,, 24	Donkey foal	3678	S. 5	,,	,,	,, 11	Aug. 26 to Sept. 9	1
	,, 24	,,	3677	I. 5	,,	,,	,, 11	Aug. 28 to Sept. 9	3
	,, 24	Horse foal	3381	S. 2	,,	,,	,, 11	Aug. 27 to 31	2
							1908.		
VII	Sept. 11	Horse	3899	S. 3	3677	6	Sept. 9	Sept. 15 to 19	3
	,, 11	,,	3894	,,	3678	6	,, 3	,, 16 to 19	4

V Generation.
VI Generation.
VI Generation,
VI Generation.

Gen.	Date of Injection.	Species.	No.	VIRUS.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.		(days)
VII	April 24	Horse	3397	S. 2	3443	6	April 21	Was not virulent	—
							1907.	1908.	
	,, 27	,,	3234	,,	2730	6	April 6	May 5 to 10	8
	,, 11	,,	3449	,,	2639	6	,, 1	April 19 to 26	8
							1908.		
VIII	,, 24	,,	3477	,,	3449	7	April 23	May 1 to 6	7

No. 2—(*continued*).

2415.

Mule 2415.
Mule 2694.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
12	15	Recovd.	–	Sept. 10	S. 3	Poly. VI	—	—	No R	Simult. 100 c.c. serum.
15	16	,,	–	—	—	—	—	—	—	
13	16	,,	–	—	—	—	—	—	—	
5	7	R †								
5	8	R D †								
4	8	R †								

Horse 2629.
Mule 2639.
Mule 2730.
Horse 3443.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
—	—	No R	–	May 7	I. 2	3449	Tzn.	7	R †	Simult. 300 c.c. serum.
5	13	R D †								
7	15	Slight R	1	April 27	S. 2	2415	Tzn.	4	R	
			2	June 19	,,	3513	CD VI	2	,,	
5	2	R D †								

R D †—Reaction with dikkop and died. R †—Reaction and died. R—Reaction.

EXPERIMENT

TYPE

III Generation,
IV Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.	1907.	(days)
V	June 20	Donkey foal	2834	I. 5	2415	4	Jan. 2	June 24 to July 3	4
VI	,, 27	Horse	2827	I. 2	2834	5	June 27	July 4 to 9	7
	Aug. 17	,,	2793	I. 5	,,	,,	,, 27	Aug. 24 to 26	7
	,, 29	Donkey foal	2564	I. 5	,,	,,	,, 27	—	—
	Sept. 2	Horse foal	2707	,,	,,	,,	,, 27	Sept. 8 to 14	—
	Oct. 4	Horse	3078	I. 10	,,	,,	June 25, 26, 27, 29	—	—
	,, 4	,,	3060	,,	,,	,,	June 25, 26, 27, 29; July 1	—	—
	,, 24	,,	3056	S. 10	,,	,,	,,	Nov. 4 to 10	10

No. 2—(*continued*).

2415.

Mules, Natal.
Mule 2415.

Re-action.	Incub. + R.	Result.		Tested.	Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1907.						
9	13	Recovd.	–	—	—	—	—	—	—	
5	12	R †								
2	9	,,								
—	—	Indef. R	–	—	—	—	—	—	—	
—	—	Irreg. R	–	Oct. 4	I. 5	2891	Tzn.	4	R †	
				1908.						
—	—	No R	1	Jan. 24	I. 2	2884	CD	3	—	Simult. 300 c.c. serum.
			2	Feb. 26	T. 10000	3375	Tzn.	18	Piropl. †	
—	—	No R	–	Oct. 24	S. 2	2891	Tzn.	4	R D †	
7	17	R †								

R†—Reaction and died. Piropl.†—Died of piroplasmosis.
RD†—Reaction with dikkop and died.

Analysis of Results—Type 2415.

"A." *Susceptibility.*

Results.

1. Of 21 subcutaneous injections into susceptible horses, 2 reacted and recovered, 15 reacted and died, 4 did not react.
2. Of 6 intrajugular injections into susceptible horses, 4 reacted and died, 2 did not react.
3. Of 30 subcutaneous injections into susceptible mules, 21 reacted and recovered, 8 reacted and died, 1 did not react.
4. Of 2 intrajugular injections into susceptible mules, 1 reacted and died, 1 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
Subcutaneous injections into horses caused	81	61	20
,, ,, ,, mules ,,	96	27	3
Intrajugular ,, ,, horses ,,	66	66	33
,, ,, ,, mules ,,	Not sufficient numbers.		

Conclusions.

1. The susceptibility of mules is greater than that of horses.
2. The mortality is greater in horses than in mules.
3. The mortality in horses is greater after an intrajugular injection than after a subcutaneous injection.

"B." *Resistance.*

1. A mule which did not react to the subcutaneous or intrajugular injection of virus, type 2415, contracted the disease later when subjected to the same type of the succeeding generation.

2. Of four horses which did not react to a subcutaneous or an intrajugular injection, two died when tested with the previous generation of the same or different type, and one reacted; one animal tested with the following generation of a different type reacted.

3. One horse which resisted the first subcutaneous injection contracted the disease and died when injected with the same dose of the same virus intrajugularly.

4. One mule which resisted (1) a subcutaneous injection and (2) an intrajugular injection proved to be susceptible to the subcutaneous injection of 2 c.c. virus of the same strain, different generation.

"C." *Virulency.*

Results.

1. Of 2 susceptible horses injected with horse virus, 1 reacted and died, 1 did not react.
2. Of 16 susceptible mules injected with horse virus, 3 reacted and recovered, 2 reacted and died, 1 did not react.
3. Of 15 susceptible horses injected with mule virus, 2 reacted and recovered, 12 reacted and died, 1 did not react.
4. Of 27 susceptible mules injected with mule virus, 18 reacted and recovered, 7 reacted and died, 2 did not react.
5. Of 10 susceptible horses injected with donkey foal virus, 6 reacted and died, 4 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
The injection of horses with horse virus caused	—	—	—
,, mules ,, ,, ,,	83	33	16
,, horses ,, mule virus caused	93	73	7
,, mules ,, ,, ,,	96	24	4
,, horses ,, donkey foal virus caused	60	60	40

Conclusions.

1. Mule virus caused the greatest percentage of reactions in mules.
2. Mule virus injected into horses caused the greatest mortality.
3. The least mortality is caused by the injection of mule virus into mules.
4. Horses show the least susceptibility to donkey virus.
5. Horse virus caused a greater percentage of mortality when injected into mules than that caused by mule virus.
6. Horse virus failed to produce reactions in mules to a greater extent than mule virus.

"*D.*" *Variability.*

4th Gen. M. 2415—100 % R. (21 animals).

5th Gen. M. 2597—43 % R. (7) | M. 2694—100 % R (4) | M. 2692—83 % R. (6) | D. F. 2834—60 % R. (5)

Conclusions.

1. The mule virus of fourth generation proved to be virulent for 100 per cent. of susceptible animals.
2. In the succeeding generation, the virulency of the virus varied from 43 to 100 per cent.
3. The generation had no influence on the virulency of the virus.

"*E.*" *Influence of dose and manner of injection.*

Injections of virus of type 2415.

1. In the dose of 2 c.c. subcutaneously, 25 reacted and recovered, 16 reacted and died, 5 did not react.
   Of these 5 non-reacters, when tested later by subcutaneous injection of 2 c.c., 2 reacted and recovered, 3 reacted and died.
2. In the dose of 3 c.c. subcutaneously, 3 reacted and died, 1 did not react.
   When tested later by a subcutaneous injection of 2 c.c., this animal reacted and recovered.
3. In the dose of 5 c.c. subcutaneously, 1 reacted and recovered.
4. In the dose of 10 c.c. subcutaneously, 1 reacted and died.
5. In the dose of 2 c.c. intrajugularly, 1 reacted and died.
6. In the dose of 5 c.c. intrajugularly, 1 reacted and died, 1 did not react.
   When tested later by intrajugular injection of 5 c.c., this animal died.
7. In the dose of 10 c.c. intrajugularly, 1 reacted and died, 1 did not react.
   When tested later by intrajugular injection of 2 c.c., this animal died.
8. In the dose of 50 c.c. intrajugularly, 1 animal reacted and died.

EXPERIMENT

With Mule Virus, Type 2891,

III Generation,
IV Generation.

Gen.	Date of Injection.	Species.	No.	Virus. Inj.	Virus. Orig.	Virus. Gen.	Virus. Date.	Reaction from.	Incub.
	1907.						1907.	1907.	(days)
V	Oct. 4	Donkey foal	2564	I. 5	2891	4	July 19	Oct. 8 to 15	4
	,, 24	Horse	3060	S. 2	,,	,,	,, 19	Nov. 1 to 6	8
	,, 4	Horse foal	2707	I. 5	,,	,,	,, 19	Oct. 11 to 16	7
	,, 4	Mule	2923	S. 2	,,	,,	,, 19	,, 13 to 18	9
	1908.								
	April 6	Horse	3409	S. 2	,,	,,	,, 19	April 13 to 19	8
	,, 6	,,	3328	,,	,,	,,	,, 19	,, 14 to 21	8
	,, 23	,,	3426	,,	,,	,,	,, 21	,, 28 to May 3	5
	1907.								
VI	Nov. 4	Horse	3095	I. 10	2707	5	Oct. 14	—	—
	Oct. 24	Mule	2958	S. 2	2564	5	Oct. 10, 12, 14	—	—
	,, 24	Donkey foal	2550	S. 10	,,	,,	,,	Nov. 1 to 8	8
	,, 24	Horse	3097	S. 10	,,	,,	,,	Oct. 30 to Nov. 4	6
VII	Nov. 8	Donkey foal	2551	S. 5	2550	6	Nov. 4	Nov. 13 to 18	5
	,, 8	Horse	3145	,,	,,	,,	,, 4	,, 14 to 18	6
VIII	,, 23	Donkey foal	2494	I. 5	2551	7	,, 17	Nov. 28 to Dec. 5	5
	1908.								
	Jan. 10	Mule	3290	S. 2	,,	,,	,, 17	Jan. 18 to 25	8

IV Generation.
V Generation,
VI Generation,
VII Generation,
VIII Generation,

Gen.	Date of Injection.	Species.	No.	Virus. Inj.	Virus. Orig.	Virus. Gen.	Virus. Date.	Reaction from.	Incub.
	1907.						1907.	1908.	(days)
IX	Dec. 27	Horse foal	2274	S. 2	2494	8	Dec. 2	Jan. 7 to 12	11
	,, 27	Donkey foal	3212	,,	,,	,,	,, 2	—	—
	1908.								
	Jan. 7	Horse foal	3231	I. 5	,,	,,	,, 2	,, 14 to 17	7
	,, 7	Donkey foal	3203	,,	,,	,,	,, 2	—	—

No. 3.

Tzaneen strain, fourth generation.

Mules, Natal.
Mule 2891.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
7	11	Recovd.	–	—	—	—	—	—	—	
5	13	R D †								
5	12	R †								
5	14	Recovd.	–	Feb. 8	I. 10	3319	P.P.R.	—	No R	
						3095	,,	—	,,	
6	14	R D †								
7	15	R & D	–	June 19	S. 2	3513	CD VI	—	R	
5	10	R D †								
				1907.						
—	—	No R	–	Dec. 19	S. 2	Mule	P.P.R.	—	R †	
				1908.						
—	—	No R	–	Feb. 8	I. 10	3319	P.P.R.	—	R D †	
						3095	,,	—	,,	
7	15	Recovd.	–	—	—	—	—	—	—	
5	11	R †								
5	10	Slight R	–	—	—	—	—	—	—	
4	10	R †								
7	12	Recovd.	–	—	—	—	—	—	—	
7	15	R †								

Mule 2891.
Donkey foal 2564.
Donkey foal 2550.
Donkey foal 2551.
Donkey foal 2494.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)									
5	16	R D †								
—	—	Indef. R	–	—	—	—	—	—	—	
3	10	R †								
—	—	Irreg. R	–	—	—	—	—	—	—	Died of pleuritis, Jan. 29, 1908.

R—Reaction. R†—Reaction and died. R & D—Reaction with dikkop.
RD†—Reaction with dikkop and died.

Analysis of Results—Type 2891.

"A." *Susceptibility.*

Results.

1. Of 7 subcutaneous injections into susceptible horses, 1 reacted and recovered, 6 reacted and died.
2. Of 3 intrajugular injections into susceptible horses, 2 reacted and died. 1 did not react.
3. Of 3 subcutaneous injections into susceptible mules, 1 reacted and recovered, 1 reacted and died, 1 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
Subcutaneous injections into horses caused	100	86	Nil.
,, ,, ,, mules ,,	67	33	33
Intrajugular ,, ,, horses ,,	67	67	33
,, ,, ,, mules ,,	—	—	—

Conclusions.

1. The susceptibility of horses is greater than that of mules.

2. The subcutaneous injection of virus, type 2891, is more fatal for horses than for mules.

3. An equal percentage of horses failed to react to an intrajugular injection, as mules failed to react to subcutaneous injection.

"B." *Resistance.*

1. A horse which resisted an intrajugular injection of 10 c.c. and a mule which resisted a subcutaneous injection of 2 c.c. both contracted the disease when tested with a different strain.

"C." *Virulency.*

Results.

1. Of 5 susceptible horses injected with mule virus, 1 reacted and recovered, 4 reacted and died.
2. One susceptible horse injected with horse virus did not react.
3. Of 4 susceptible horses injected with donkey foal virus, 4 reacted and died.
4. Of 2 susceptible mules injected with donkey foal virus, 1 reacted and died, 1 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
The injection of horses with mule virus caused	100	80	—
,, ,, ,, donkey foal virus caused	100	100	—

Conclusions.

1. Mule and donkey foal virus each caused the maximum percentage of reactions.

2. Donkey foal virus is more fatal than mule virus.

"E." Influence of dose and manner of injection.

Injections of virus of type 2891.

1. In the dose of 2 c.c. subcutaneously, 2 reacted and recovered, 7 reacted and died, 1 did not react.
   When tested later by intrajugular injection of 10 c.c. this animal reacted and died.

2. In the dose of 5 c.c. intrajugularly, 2 reacted and died.

3. In the dose of 10 c.c. intrajugularly, 1 did not react.
   When tested later by subcutaneous injection of 2 c.c. (different strain) this animal reacted and died.

EXPERIMENT

With Mule Virus, Type 2539,

HORSE 1087, TZANEEN

I Generation,
II Generation,
III Generation,
IV Generation.

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.		(days)
V	Jan. 22	Mule	2539	S. 2	2415	4	Jan. 2	Jan. 26 to Feb. 2	3
VI	Feb. 4	,,	2495	,,	2539	5	,, 30	Feb. 14 to 21	10
	,, 27	,,	2669	,,	,,	,,	,, 30	Was not virulent	—
	Mar. 20	,,	2693	,,	,,	,,	,, 28	,,	—
	,, 20	,,	2694	,,	,,	,,	,, 28	,,	—
	,, 20	,,	2695	,,	,,	,,	,, 30	,,	—
	,, 20	,,	2696	,,	,,	,,	,, 30	,,	—
	,, 28	Horse	2722	,,	,,	,,	,, 30	—	—
	1908.								
	Jan. 10	Mule	3227	,,	,,	,,	,, 30	—	—
	Feb. 17	Horse	3248	S. 50	,,	,,	,, 30	Feb. 26 to Mar. 1	9

No. 4.

Tzaneen strain, fifth generation.

(Origin).

Mule 1965.
Mule 1996.
Mule (Natal) 116/97.
Mule 2415.

Reaction.	Incub. + R.	Result.	Tested.	Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)		1907.						
7	10	Recovd.	1 Feb. 5	S 2	2407	Ord.	38	Reaction	
			2 April 16	,,	2406	OTBLPW	—	,,	
7	17	,,	1 Feb. 26	,,	2598	Ord.	38	R ?	
			2 April 17	,,	2406	OTBLPW	—	No R	
					2709	Ord.	—		
			3 May 3	S 6	2148	Tzn.	—	,,	
					2168	Bul.	—		
—	—	R ?	– Mar. 20	S. 5	2415	Tzn.	4	Reaction	
—	—	,,	1 April 4	S. 2	2629	,,	5	,,	
			2 ,, 23	,,	2406	OTBLPW	—	Slight R	Simult. 100 c.c. serum.
					2709	Ord.	—		
			3 May 14	S. 5	2148	Tzn.	—	No R	
					2168	Bul.	—		
—	—	,,	1 April 4	S. 2	2415	Tzn.	4	R & D	
			2 ,, 23	,,	2406	OTBLPW	—	R ?	Simult. 100 c.c. serum.
					2709	Ord.	—		
			3 May 14	S. 5	2148	Tzn.	—	No R	
					2168	Bul.	—		
—	—	,,	1 April 4	S. 2	2629	Tzn.	5	Reaction	
			2 ,, 23	,,	2406	OTBLPW	—	Slight R	Simult. 100 c.c. serum.
					2709	Ord.	—		
			3 May 14	S. 5	2148	Tzn.	—	No R	
					2168	Bul.	—		
—	—	,,	1 April 4	S. 2	2415	Tzn.	4	Reaction	
			2 ,, 23	,,	2406	OTBLPW	—	Slight R	Simult. 100 c.c. serum.
					2709	Ord.	—		
			3 May 14	S. 5	2148	Tzn.	—	No R	
					2168	Bul.	—		
—	—	No R	– April 16	I. 2	2539	Tzn.	5	,,	Died of piroplasma, 25/5/07.
			1908.						
—	—	,,	– Jan. 28	S. 2	2415	Tzn.	4	R D †	
4	13	R †							

R?—Doubtful reaction. R†—Reaction and died. R & D—Reaction with dikkop.
RD†—Reaction with dikkop and died.

EXPERIMENT

TYPE

IV Generation,
V Generation,
VI Generation,
VI Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.	1907.	(days)
VII	Mar. 30	Horse	2705	S. 2	2495	6	Feb. 19	April 7 to 13	7
	1908.							1908.	
	Feb. 26	Mule	3288	,,	,,	,,	,, 19	March 10 to 16	13
	Mar. 20	,,	3434	,,	,,	,,	,, 19	1908.	—
	,, 20	Horse	3437	,,	,,	,,	,, 19	—	—
	April 23	,,	3439	,,	3248	6	,, 29	April 30 to May 4	7

VI Generation,
VII Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1907.	1908.	(days)
VIII	April 27	Horse	3342	S. 2	2705	7	April 12	May 4 to 10	7
							1908.		
	,, 6	,,	3396	,,	3288	7	Mar. 11	—	—
	,, 20	,,	3496	S. 50	,,	,,	,, 11	April 27 to May 3	7
	,, 20	,,	3498	S. 2	,,	,,	,, 11	—	—
	May 14	,,	3540	S. 5	,,	,,	,, 11	—	—
	June 3	—	—	I. 5	,,	,,	,, 11	—	—
	,, 26	—	—	I. 10	,,	,,	,, 11	July 1 to 4	4
	May 21	Horse	3553	I. 2	,,	,,	,, 11	—	—
	June 3	Mule	3528	I. 2	,,	,,	,, 11	—	—
	,, 26	—	—	I. 10	,,	,,	,, 11	July 2 to 9	6
	,, 9	Mule	3564	S. 2	,,	,,	,, 11	—	—

No. 4—(*continued*).

2539.

Mule 2415.
Mule 2539.
Mule 2495.
Horse 3248.

Re-action.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days) 6	(days) 13	R †								
				1908.						
6	19	Recovd.	–	May 7	S. 2	3494	P.P.R.	1	R	
—	—	R ?	1	April 18	,,	3446	Tzn.	8	R ?	
			2	May 7	,,	3375	,,	18	No R	
			3	June 2	S. 8	—	Polyv. virus mixt.	—	,,	Simult. 50 c.c. serum.
—	—	,,	1	April 18	S. 2	3446	Tzn.	8	R	
			2	May 7	,,	3375	,,	18	No R	Simult. 200 c.c. serum.
			3	June 11	S. 10000	3609	,,	19	R †	
4	11	R †								

Mule 2495.
Horse 2705.
Mule 3288.

Re-action.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks
(days) 6	(days) 13	R †								
				1908.						
—	—	No R	–	April 23	S. 2	3368	Tzn.	5	R †	
6	13	R †								
—	—	No R	–	May 12	,,	2415	,,	4	,,	
—	—	,,	–	—	—	—	—	—	—	
—	—	,,	–	—	—	—	—	—	—	
4	8	R †								
—	—	No R	1	June 5	S. 2	3361	Tzn.	7	Reaction	
			2	,, 30	,,	3619	,,	20	,,	On the 9th day 200 c.c. serum.
—	—	No R	–	—	—	—	—	—	—	
7	13	R †								
—	—	No R	1	June 19	S. 20	3359	Tzn.	6	Reaction	
			2	July 31	S. 2	3699	,,	6	No R	
			3	Aug. 24	S. 3	—	Polyv. VI	—	Slight R	After 24 hrs. 100 c.c. serum.

R—Reaction. R†—Reaction and died. R ?—Doubtful reaction.

EXPERIMENT

TYPE

VI Generation,
VII Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.	1908.	(days)
VIII	April 6	Horse	3446	S. 2	3434	7	April 2	April 12 to 18	5
	,, 20	,,	3480	,,	,,	,,	,, 2	—	—
	May 7	—	—	S. 10	,,	,,	,, 2	—	—
	,, 21	—	—	I. 10	,,	,,	,, 2	—	—
	April 20	Horse	3492	S. 2	,,	,,	,, 2	—	—
	May 7	—	—	S. 10	,,	,,	,, 2	—	—
	,, 21	—	—	I. 10	,,	,,	,, 2	—	—
	,, 21	Horse	3548	I. 2	,,	,,	,, 2	May 26 to 30	4
	June 3	Mule	3525	,,	,,	,,	,, 2	—	—
	,, 26	—	—	I. 10	,,	,,	,, 2	—	—
	,, 9	Mule	3565	S. 2	,,	,,	,, 2	June 19 to 24	9
	July 4	Donkey foal	3577	I. 5	,,	,,	,, 2	—	—

VII Generation,
VIII Generation,
VIII Generation,
VIII Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.	1908.	(days)
IX	April 18	Horse	3437	S. 2	3446	8	April 16	April 24 to May 3	5
	,, 23	,,	3341	,,	,,	,,	,, 16	April 28 to May 3	4
	June 23	,,	3670	,,	3565	8	June 22	—	—
	,, 23	Mule	3653	,,	,,	,,	,, 22	—	—
	Aug. 8	Horse	3752	S.10	,,	,,	,, 22	—	—
	,, 8	,,	3753	I. 10	,,	,,	,, 22	—	—

No. 4—(*continued*).

2539.

Mule 2495.
Mule 3434.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	days)									
7	12	R †								
—	—	No R	–	—	—	—	—	—	—	
—	—	,,	–	—	—	—	—	—	—	
				1908.						
—	—	,,	–	June 3	S. 2	3361	Tzn.	7	R †	
—	—	,,	–	—	—	—	—	—	—	
—	—	,,	–	—	—	—	—	—	—	
—	—	,,	–	June 3	S. 2	3361	Tzn.	7	R †	
5	9	R †								
—	—	No R	–	—	—	—	—	—	—	
—	—	,,	–	July 31	S. 2	3699	Tzn.	6	R †	
6	15	Recovd.	–	June 30	S. 3	—	Polyv. VI	—	,,	
—	—	R ?								

Mule 3434.
Horse 3446.
Mule 3565.
Donkey Foal 3577.

Reaction.	Incub. + R.	Result.		Tested.	Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
10	15	Recovd.	1	May 7	S. 2	3375	Tzn.	18	No R	Simult. 200 c.c. serum.
			2	June 11	10000	3609	,,	19	R †	
6	10	R †								
—	—	No R.	–	July 14	S. 3	—	Polyv. VI	—	R †	Simult. 400 c.c. serum.
—	—	,,	–	,, 31	S. 2	3699	Tzn.	6	R †	
—	—	,,	–	Sept. 1	S. 3	3788	,,	6	No R	Simult. 300 c.c. serum.
			–	Oct. 8	T. 10	1391 1392	—	—	R	
—	—	No R	–	Sept. 1	S. 3	3788	Tzn.	6	No R	24 hrs. later 300 c.c. serum.
			–	,, 15	S. 2	—	Polyv. VI	—	R †	Simult. 300 c.c. serum.

R—Reaction. R †—Reaction and died. R ?—Doubtful reaction.

Analysis of Results—Type 2539.

"A." *Susceptibility.*

Results.

1. Of 17 subcutaneous injections into susceptible horses, 1 reacted and recovered, 7 reacted and died, 9 did not react.
2. Of 6 intrajugular injections into susceptible horses, 2 reacted and died, 4 did not react.
3. Of 10 subcutaneous injections into susceptible mules, 4 reacted and recovered, 6 did not react.
4. Of 4 intrajugular injections into susceptible mules, 1 reacted and died, 3 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
Subcutaneous injections into horses caused	46	40	70
,, ,, ,, mules ,,	40	Nil	60
Intrajugular ,, ,, horses ,,	33	33	66
,, ,, ,, mules ,,	25	25	75

Conclusions.

1. Horses were more susceptible than mules.
2. The subcutaneous injection of virus, type 2539, is more fatal for horses than for mules.
3. The intrajugular injection of virus, type 2539, is more fatal for horses than for mules.
4. The intrajugular injection into horse or mule is more fatal than the subcutaneous injection.
5. The number of susceptible animals which proved refractory to the injection of virus, type 2539, is greater than the number of animals which reacted.

"B." *Resistance.*

1. Of 4 mules injected subcutaneously or intrajugularly with virus, type 2539, and tested later with the same type, 3 reacted and recovered and 1 died.
2. Of 3 mules injected subcutaneously or intrajugularly with virus, type 2539, and tested later with a different type, all reacted and recovered.
3. Of 3 horses injected subcutaneously or intrajugularly with virus, type 2539, 1 reacted and recovered, 1 reacted and died, and 1 did not react to the subsequent test, and later proved to be immune.
4. Of 2 horses injected subcutaneously or intrajugularly with virus, type 2539, and tested later with a different type, 1 reacted and died and 1 reacted and recovered.
5. One horse injected subcutaneously and intrajugularly in the dose of 5 c.c. virus, type 2539, did not react, but died later when injected with 10 c.c. virus, same type.
6. One mule injected subcutaneously with 2 c.c. virus, type 2539, did not react, and died later when injected intrajugularly with 10 c.c. same virus.
7. Two horses injected subcutaneously in the dose of 2 and 10 c.c., followed by an intrajugular injection of 10 c.c. virus, type 2539, did not react, but both died later when injected with the same strain of virus, different type.
8. One mule injected intrajugularly in the dose of 2 and 10 c.c. did not react, but died when tested later with the same strain of virus, different type.
9. The blood of 1 mule which had a typical reaction was not virulent for 3 susceptible horses and 1 mule.

"C." *Virulency.*

Results.

1. Of 4 susceptible horses injected with horse virus, 1 reacted and recovered, 3 reacted and died.
2. Of 13 susceptible horses injected with mule virus, 6 reacted and died, 7 did not react.
3. Of 14 susceptible mules injected with mule virus, 4 reacted and recovered, 1 reacted and died, 9 did not react.

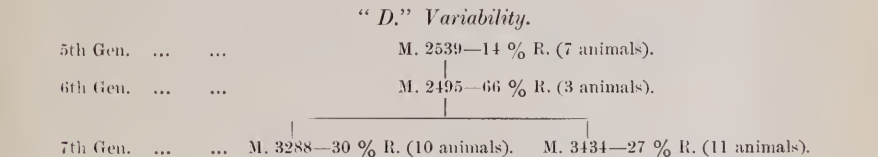
Taking these figures out according to percentages,

	Reactions. %	Deaths. %	No Reaction %
The injection of susceptible horses with horse virus caused	100	75	Ni.
The injection of susceptible horses with mule virus caused	44	44	56
The injection of susceptible mules with mule virus caused	37	9	63

Conclusions.

1. Horse virus injected into susceptible horses caused the greatest percentage of reactions.
2. The injection of mule virus is more fatal for horses than for mules.
3. The least mortality was caused by the injection of mule virus into mules.

"D." *Variability.*



Conclusions.

The virulency of the strain is but slightly pronounced, yet the influence of an animal through which it passed becomes evident in the second generation and in the last generation; in the former increasing and in the last disappearing completely.

"E." *Influence of dose and manner of injection.*

Injections of virus of type 2539.

1. In the dose of 2 c.c. subcutaneously, 5 reacted and recovered, 5 reacted and died, 12 did not react.
   Of these 12 non-reacters, 1 was tested later by subcutaneous injection of 5 c.c., this animal reacted and recovered; 10 were tested subcutaneously with 2 c.c. virus, 5 reacted and recovered, 5 reacted and died.
   One tested with 20 c.c. intrajugularly, reacted and died.
2. In the dose of 5 c.c. subcutaneously, 1 did not react. When tested later by intrajugular injection of 10 c.c., this animal reacted and died.
3. In the dose of 50 c.c. subcutaneously, 2 reacted and died.
4. In the dose of 2 c.c. intrajugularly, 1 reacted and died, 3 did not react.
   Of these 3 non-reacters, 2 were tested later by a subcutaneous injection of 3 c.c., 1 reacted and recovered, 1 reacted and died.
   One was tested with 10 c.c. intrajugularly, and reacted and died.

EXPERIMENT

With Mule Virus, Type 2694,

HORSE 1087, TZANEEN

I Generation.
II Generation.
III Generation,
IV Generation.

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1907.						1907.	1907.	(days)
V	April 4	Mule	2694	S. 2	2415	4	Jan. 2	April 9 to 18	5
	1908.							1908.	
VI	Feb. 26	,,	3359	,,	2694	5	April 11	March 5 to 12	7
							1908.		
VII	April 15	Horse	3385	,,	3359	6	March 9	—	—
	,, 22	,,	3464	,,	,,	,,	,, 9	April 30 to May 9	8
	,, 23	,,	3484	,,	,,	,,	,, 9	—	—
	,, 27	,,	3478	,,	,,	,,	,, 9	—	—
	May 8	,,	3264	,,	,,	,,	,, 11	—	—
	,, 21	—	—	I. 2	,,	,,	,, 11	May 26 to 31	5
	,, 8	Horse	3270	,,	,,	,,	,, 11	May 14 to 21	6
	June 15	,,	3626	S. 2	,,	,,	,, 11	—	—
	,, 15	,,	3356	S. 10	,,	,,	,, 11	—	—
	,, 15	,,	3364	S. 50	,,	,,	,, 11	—	—
	,, 15	,,	3406	S. 20	,,	,,	,, 11	June 28 to July 3	13
	,, 15	Donkey foal	3578	I. 10	,,	,,	,, 11	June 24 to 30	9
	,, 19	Mule	3564	S. 20	,,	,,	,, 11	June 23 to July 1	4
	,, 19	,,	3650	I. 20	,,	,,	,, 11	June 23 to 29	4
	July 4	Donkey foal	3711	I. 5	,,	,,	,, 11	July 16 to 27	12
	,, 4	,,	3712	,,	,,	,,	,, 11	,, 18 to 30	14
	,, 31	,,	3661	,,	,,	,,	,, 11	Aug. 4 to 17	4
	,, 31	,,	3676	,,	,,	,,	,, 11	,, 3 to 16	3

No. 5.

Tzaneen strain, fifth generation.

(ORIGIN).

Mule 1965.
Mule 1996.
Mule (Natal) 116. 1897.
Mule 2415.

Reaction.	Incub. + R.	Result.	Tested.		Virus.				Result.	Remarks.
					Inj.	Orig.	Qual.	Gen.		
(days)	(days)			1907.						
9	14	R & D	1	April 23	S. 2	2406	OTBLPW	—	No R	Simult. 100 c.c. serum.
			2	May 14	S. 5	2709	Ord.	—	,,	
						2148	Tzn.	—	,,	
						2168	Bul.	—	,,	
				1908.						
8	15	Recovd.	–	May 7	S. 2	3494	P.P.R.	1	Slight R	
					,,	3501	CD VI	1	,,	
—	—	Irreg. R	–	April 27	,,	2891	Tzn.	4	R †	
9	17	R & D	–	—	—	—	—	—	—	Was killed later.
—	—	Irreg. R	–	May 14	S. 2	2415	,,	4	R †	
—	—	No R	1	May 21	,,	3361	,,	7	No R	Simult. 200 c.c. serum.
			2	June 26	,,	3509	,,	8	Reaction	After 3 days 300 c.c. serum.
			3	July 29	,,	3749	,,	21	,,	After 3 days 200 c.c. serum.
—	—	No R	–	—	—	—	—	—	—	
5	10	R †								
7	13	,,								
—	—	No R	1	July 15	S. 2	3557	Tzn.	8	R ?	After 3 days 200 c.c. serum.
			2	,, 23	S. 3	Poly. VI	—	—	R †	Simult. 200 c.c. serum.
—	—	R ?	–	,, 21	S. 2	3398	Tzn.	6	Reaction	
—	—	No R	–	,, 15	,,	3570	,,	8	R †	
5	18	R †								
6	15	Recovd.	–	—	—	—	—	—	—	
8	12	Recovd.	–	July 31	S. 2	3699	Tzn.	6	No R	
6	10	R †	–							
11	23	Recovd.	–	—	—	—	—	—	—	
12	26	,,	–	—	—	—	—	—	—	
13	17	,,		—	—	—	—	—	—	Died of poverty, Aug. 20, 1908.
13	16	,,	–	—	—	—	—	—	—	

R—Reaction. R & D—Reaction with dikkop. R †—Reaction and died.

EXPERIMENT

TYPE

IV Generation,
V Generation,
VI Generation.
VII Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.	1908.	(days)
VIII	May 12	Horse	3506	S. 2	3464	7	May 8	—	—
	June 3			I. 2	,,	,,	,, 8	June 8 to 12	5
	May 29	Horse	3570	S. 2	,,	,,	,, 8	,, 6 to 14	8
	,, 29	,,	3569	,,	,,	,,	,, 8	—	—
	,, 29	,,	3557	S. 5	,,	,,	,, 8	June 9 to 16	11
	,, 29	,,	3579	S. 10	,,	,,	,, 8	—	—
	,, 29	,,	3516	I. 2	,,	,,	,, 8	,, 2 to 8	4
	June 11	Mule	3592	I. 10	,,	,,	,, 8	,, 18 to 26	7
	,, 11	Donkey foal	3576	,,	,,	,,	,, 8	—	—
	,, 15	Horse	3625	S. 2	,,	,,	,, 8	—	—
	,, 15	,,	3629	,,	,,	,,	,, 8	—	—
	,, 15	,,	3632	,,	,,	,,	,, 8	—	—
	,, 19	,,	3603	I. 0·02	,,	,,	,, 8	—	—
	July 1	,,	3644	S. 2	,,	,,	,, 8	—	—
	,, 21			I. 2	,,	,,	,, 8	July 27 to Aug. 3	6
	,, 4	Horse	3541	S. 10	,,	,,	,, 8	—	—
	,, 21			I. 2	,,	,,	,, 8	—	—
	Aug. 14	Mule	3793	S. 5	,,	,,	,, 8	Aug. 20 to 29	5
	,, 14	,,	3810	,,	,,	,,	,, 8	—	—
	,, 14	Horse	3819	,,	,,	,,	,, 8	,, 21 to 31	6
	,, 14	,,	3822	,,	,,	,,	,, 8	—	—
	,, 14	,,	3823	,,	,,	,,	,, 8	—	—

No. 5—(*continued*).

2694.

Mule 2415.
Mule 2694.
Mule 3359.
Horse 3464.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
—	—	No R	–	—	—	—	—	—	—	
4	9	R †								
8	16	,,								
—	—	Irreg. R	–	June 26	S. 2	3361	Tzn.	7	Irreg. R	
7	18	R †								
—	—	Irreg. R	1	,, 26	,,	,,	,,	7	No R	
			2	July 29	,,	3749	,,	21	R †	Simult. 200 c.c. serum.
6	10	R †								
8	15	Recovd.	–	June 30	S. 3	Poly. VI	—	—	R †	
—	—	Irreg. R	–	—	—	—	—	—	—	
—	—	R ?	–	July 4	S. 2	3361	Tzn.	7	R †	
—	—	,,	–	,, 4	,,	,,	,,	,,	,,	
—	—	No R	1	,, 4	,,	3433	,,	8	No R	
			2	,, 21	I. 2	,,	,,	,,	R †	
—	—	,,	–	,, 15	S. 2	3557	,,	,,	,,	Simult. 300 c.c. mule serum.
—	—	R ?	–	—	—	—	—	—	—	
7	13	R †								
—	—	No R	–	—	—	—	—	—	—	
—	—	,,	–	Aug. 11	S. 1	3557	Tzn.	8	R	
						3270	,,	7	,,	
10	15	Recovd.	–	Sept. 10	S. 3	—	Polyv. VI	—	No R	Simult. 100 c.c. serum.
—	—	No R	–	,, 10	,,	—	,,	—	R †	,, ,, ,,
11	17	R & D	1	Sept. 9	,,	—	,,	—	No R	Simult. 300 c.c. serum.
			2	Oct. 14	T.2250	3934	P.P.R.	—	No R	
—	—	No R		Sept. 9	S. 3	—	Polyv. VI	—	R †	,, ,, ,,
—	—	,,	1	,, 1	,,	3793	Tzn.	8	No R	After 24 hrs. 300 c.c. serum.
			2	,, 15	S. 2	—	Polyv. VI	—	,,	Simult. 300 c.c. serum.

R—Reaction. R?—Doubtful reaction. R†—Reaction and died.
R & D—Reaction with dikkop.

EXPERIMENT

TYPE

VI Generation,
VII Generation,
VII Generation,
VII Generation,
VII Generation,
VII Generation,
VII Generation,
VII Generation,

Gen.	Date of Injection.	Species.	No.	VIRUS.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.	1908.	(days)
VIII	July 1	Horse	3726	S. 2	3270	7	May 20	July 8 to 14	7
	,, 1	,,	3729	,,	3406	,,	June 30	,, 7 to 12	6
	,, 15	,,	3741	,,	3356	,,	July 3	—	—
	,, 1	,,	3717	,,	3564	,,	June 28	—	—
	,, 21			I. 10	,,	,,	,, 28	July 27 to Aug. 3	6
	Aug. 14	Horse	3738	S. 3	,,	,,	,, 28	—	—
	,, 14	,,	3830	,,	,,	,,	,, 28	Aug. 23 to 31	8
	July 1	,,	3720	S. 2	3650	,,	,, 28	July 6 to 10	4
	,, 4	,,	3265	,,	,,	,,	,, 28	,, 8 to 12	4
	,, 22	,,	3754	I. 5	3711	,,	July 21	July 28 to Aug. 6	6
	,, 29	,,	3682	S. 5	,,	,,	,, 21	—	—
	,, 22	,,	3756	S. 5	3712	,,	July 21	July 29 to Aug. 4	6
	,, 22	,,	3757	I. 5	,,	,,	,, 21	July 29 to Aug. 2	6

No. 5—(*continued*).

2694.

Mule 3359.
Horse 3270.
Horse 3406.
Horse 3356.
Mule 3564.
Mule 3650.
Donkey foal 3711.
Donkey foal 3712.

Reaction.	Incub. + R.	Result.		Tested.	Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
6	13	R †								
5	11	,,								
—	—	R ?	–	Aug. 14	S. 3	3592	Tzn.	8	R D †	
—	—	No R								
7	13	R †								
—	—	No R	–	Sept. 9	S. 3	—	Polyv. VI	—	R †	Simult. 300 c.c. serum.
9	17	R & D	–	,, 9	,,	—	,,	—	No R	,, ,, ,,
5	9	R †								
4	8	,,								
5	11	,,								
—	—	R ?	–	—	—	—	—	—	—	Killed on account of debility, Sept. 18, 1908.
7	13	R †								
5	11	,,								

R—Reaction. R?—Doubtful reaction. R†—Reaction and died. R & D—Reaction with dikkop. RD†—Reaction with dikkop and died.

EXPERIMENT

TYPE

VI Generation,
VII Generation,
VII Generation,
VII Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.	1908.	(days)
VIII	July 1	Horse	3723	S. 2	3578	7	June 29	—	—
	,, 21			I. 2	,,	,,	,, 29	—	—
	,, 4	Horse	3737	S. 5	,,	,,	,, 29	July 12 to 22	7
	,, 4	,,	3239	S. 2	,,	,,	,, 29	—	—
	,, 21			I. 2	,,	,,	,, 29	—	—
	,, 4	Horse	3268	S. 2	,,	,,	,, 29	—	—
	,, 21			I. 2	,,	,,	,, 29	—	—
	,, 31	Horse	3767	S. 5	,,	,,	,, 29	—	—
	,, 31	,,	3769	,,	,,	,,	,, 29	—	—
	,, 31	,,	3782	,,	,,	,,	,, 29	—	—
	Aug. 14	Horse foal	3594	S. 2	,,	,,	,, 29	—	—
	,, 14	,,	3597	,,	,,	,,	,, 29	Aug. 20 to 26	5
	,, 14	Horse	3813	S. 3	3661	,,	Aug. 11	,, 21 to Sept. 2	6
	,, 14	,,	3815	,,	3676	,,	,, 11	Aug. 20 to 26	5

No. 5—(*continued*).

2694

Mule 3359.
Donkey foal 3578.
Donkey foal, 3661.
Donkey foal 3676.

Reaction.	Incub. + R.	Result.	Tested.		Virus.				Result.	Remarks.
					Inj.	Orig.	Qual.	Gen.		
(days)	(days)			1908.						
—	—	No R	–	—	—	—	—	—	—	
—	—	R ?	1	Aug. 11	S. 1	3557	Tzn.	8	No R	Simult. 300 c.c. serum.
						3270	,,	7	,,	
			2	Sept. 9	S. 3	—	Polyv. VI	—	R	,, ,, ,,
11	18	,,	–	July 29	S. 2	3650	Tzn.	7	R †	
—	—	,,	–	—	—	—	—	—	—	
—	—	No R	1	Aug. 11	S. 1	3557	Tzn.	8	R & D	Simult. 300 c.c. serum.
						3270	,,	7	,,	
			2	,, 27	S. 3	—	Polyv. VI	—	Slight R	,, ,, ,,
—	—	No R	–	—	—	—	—	—	—	
—	—	,,	–	,, 11	S. 1	3557	Tzn.	8	R D †	,, ,, ,,
						3270	,,	7	,,	
—	—	No R	1	,, 11	,,	3557	,,	8	No R	,, ,, ,,
						3270	,,	7		
			2	Oct. 13	S. 2	—	Polyv. VI	—	,,	,, ,, ,,
—	—	,,	–	Aug. 11	S. 1	3557	Tzn.	8	,,	,, ,, ,,
						3270	,,	7	,,	Died of debility, Oct. 14, 1908.
—	—	,,	1	,, 11	,,	3557	,,	8	,,	Simult. 300 c.c. serum.
						3270	,,	7	,,	
			2	Sept. 9	S. 3	—	Polyv. VI	—	R D †	,, ,, ,,
—	—	R ?	–	—	—	—	—	—	—	
7	12	R †								
13	19	R & D	–	Sept. 9	S. 3	—	Polyv. VI	—	R	,, ,, ,,
7	12	R †								

R—Reaction. R?—Doubtful reaction. R†—Reaction and died. R & D—Reaction with dikkop. RD†—Reaction with dikkop and died.

Gen.	Date of Injection.	Species.	No.	Virus. Inj.	Orig.	Gen.	Date.	Reaction from.	Incub.	Experiment Type
	1908.						1908.	1908.	(days)	
IX	June 15	Horse	3628	S. 2	3569	8	June 8	—	—	VI Generation.
	July 4			S. 10	,,	,,	,, 8	July 8 to 12	4	VII Generation.
	June 15	Horse	3633	,,	,,	,,	,, 8	—	—	VIII Generation.
	July 4			I. 2	,,	,,	,, 8	—	—	VIII Generation.
	,, 21			,,	,,	,,	,, 8	—	—	VIII Generation.
	June 23	Horse	3646	S. 2	3592	8	June 22	—	—	VIII Generation.
	,, 23	,,	3647	I. 2	,,	,,	,, 22	June 28 to July 4	5	
	Aug. 14	,,	3741	S. 3	,,	,,	,, 22	Aug. 23 to Sept. 3	8	
	,, 14	,,	3747	,,	,,	,,	,, 22	—	—	
	June 26	,,	3710	S. 5	3576	,,	,, 24	—	—	
	July 21			I 2	,,	,,	,, 24	—	—	
	,, 15	Horse	3364	S. 2	3570	8	,, 11	July 19 to 25	4	

Gen.	Date of Injection.	Species.	No.	Virus. Inj.	Orig.	Gen.	Date.	Reaction from.	Incnb.	Experiment Type
	1908.						1908.	1908.	(days)	
IX	July 22	Horse	3758	S. 2	3737	8	July 18	July 27 to 30	5	VI Generation.
	Aug. 14	,,	3613	I. 10	,,	,,	,, 18	—	—	VII Generation.
	July 29	,,	3617	S. 10	3741	9	,, 28	—	—	VII Generation.
	Aug. 24	,,	3859	S. 2	3597	8	Aug. 21	—	—	VIII Generation.
										VIII Generation.
										VIII Generation.
										VIII Generation.

No. 5—(*continued*).

2694.

Mule 3359.
Horse 3464.
Horse 3579.
Horse 3569.
Mule 3592.
Donkey foal 3576.
Horse 3570.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
—	—	No R								
4	8	R †								
—	—	No R								
—	—	,,								
—	—	,,	–	Aug. 11	S. 1	3557	Tzn.	8	R †	Simult. 300 c.c. serum.
						3270	,,	7	,,	
—	—	,,	–	July 14	S. 3	—	Polyv. VI	—	,,	Simult. 400 c.c. polyv. serum.
6	11	R †								
12	20	R D †								
—	—	No R	–	Sept. 9	S. 3	—	Polyv. VI	—	R D †	Simult. 300 c.c. serum.
—	—	R ?								
—	—	No R	–	Aug. 14	S. 2	3749	Tzn.	21	R †	After 2 days 200 c.c. serum.
6	10	R D †								

Mule 3359.
Donkey foal 3578.
Horse 3464.
Horse 3737.
Horse 3741.
Horse foal 3594.
Horse foal 3597.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
3	8	R †								
—	—	No R	–	Sept. 9	S. 3	—	Polyv. VI	—	No R	Simult. 300 c.c. serum.
—	—	,,	1	Aug. 14	,,	3592	Tzn.	8	,,	
						3564	,,	7	,,	
			2	Sept. 9	,,	—	Polyv. VI	—	R †	,, ,, ,,
—	—	,,	–	,, 11	,,	3678	Tzn.	6	R †	3 Days later, 300 c.c. serum.

R—Reaction. R?—Doubtful reaction. R†—Reaction and died. RD—Reaction with dikkop. RD†—Reaction with dikkop and died.

Analysis of Results—Type 2694.

"A." *Susceptibility.*

Results.

1. Of 51 subcutaneous injections into susceptible horses, 4 reacted and recovered, 14 reacted and died, 33 did not react.
2. Of 17 intrajugular injections into susceptible horses, 9 reacted and died, 8 did not react.
3. Of 5 subcutaneous injections into susceptible mules, 4 reacted and recovered, 1 did not react.
4. Of 2 intrajugular injections into susceptible mules, 1 reacted and recovered, 1 reacted and died.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
Subcutaneous injections into horses caused	34	26	64
,, ,, ,, mules ,,	80	Nil	20
Intrajugular ,, ,, horses ,,	52	52	48
,, ,, ,, mules ,,	Not sufficient numbers.		

Conclusions.

1. The susceptibility of mules is greater than that of horses.
2. The subcutaneous injection of virus, type 2694, is more fatal for horses than for mules.
3. The intrajugular injection into horse or mule is more fatal than the subcutaneous injection.
4. The subcutaneous injection was resisted to a greater extent by horses than by mules.

"B." *Resistance.*

1. Four horses which did not react to the subcutaneous or intrajugular injection of virus, type 2694, all died when tested with the same type.
2. Of 14 horses which did not react to the subcutaneous or intrajugular injection of virus, type 2694, 9 died and 5 reacted when tested with the same strain, different type.
3. Of 8 horses which did not react to the subcutaneous or intrajugular injection of the virus, type 2694, 1 reacted and 7 died when tested later with a different strain of virus.
4. One mule which did not react to the subcutaneous injection died when tested later with a different strain.
5. Three horses which did not react to the subcutaneous injection of 2 c.c. virus died when injected intrajugularly with the same dose of the same virus.
6. Two horses which did not react to the subcutaneous injection of 2 c.c. virus died when injected intrajugularly with 10 c.c. same virus.

7. One horse which did not react to a subcutaneous injection of 10 c.c. nor to an intrajugular injection of 2 c.c. gave a reaction when tested with the same strain, different type.

8. One horse which did not react to a subcutaneous injection nor to an intrajugular injection of 2 c.c., and which did not react to a subcutaneous injection of 1 c.c. virus of a different type, gave a reaction when injected subcutaneously with 3 c.c. virus, different strain.

9. Of two horses which did not react to a subcutaneous or an intrajugular injection of 2 c.c. virus, one reacted and the other died when tested with virus of a different type.

10. One horse which did not react to a subcutaneous injection and two intrajugular injections died when tested with a different type, same strain.

11. One horse which did not react to a subcutaneous injection of 2 c.c. virus died when tested with the same virus, different type.

"C." *Virulency.*

Results.

1. Of 25 susceptible horses injected with horse virus, 1 reacted and recovered, 10 reacted and died, 14 did not react.
2. Of 3 susceptible mules injected with horse virus, 2 reacted and recovered, 1 did not react.
3. Of 19 susceptible horses injected with mule virus, 2 reacted and recovered, 8 reacted and died, 9 did not react.
4. Of 4 susceptible mules injected with mule virus, 3 reacted and recovered, 1 reacted and died.
5. Of 16 susceptible horses injected with donkey foal virus, 1 reacted and recovered, 5 reacted and died, 10 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
The injection of horses with horse virus caused	44	40	56
,, ,, mules ,, ,, ,, ,,	67	—	33
,, ,, horses ,, mule ,, ,,	55	45	45
,, ,, mules ,, ,, ,, ,,	100	25	Nil.
,, ,, horses ,, donkey foal virus caused	40	34	60

Conclusions.

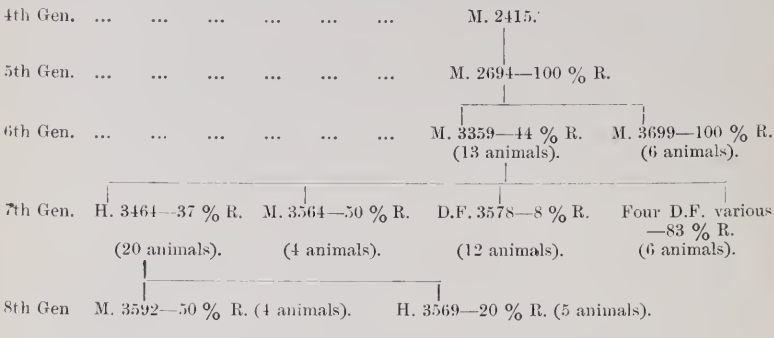
1. The injection of mule virus caused the greatest percentage of reactions.
2. The injection of mule virus caused the greatest mortality.
3. The least mortality and the least reactions were caused by the injection of donkey foal virus.

4. Horse virus failed to cause reactions in a greater percentage of horses than in mules.

5. Mule virus failed to cause reaction in a greater percentage of horses.

6. Donkey virus failed to produce reactions in the greatest percentage.

"*D.*" *Variability.*



Conclusions.

1. The virulency of virus is not affected by the generation. It varies within the same generation between the horses, mules, and donkeys, and again between the various lots of donkeys and two different mules.

2. It is remarkable that the virus of the mule caused more reactions than that of horses.

3. The virulency was particularly affected by mule 3699.

"*E.*" *Influenee of dose and manner of injection.*

Injections of virus of type 2694.

1. In the dose of 2 c.c. subcutaneously, 3 reacted and recovered, 8 reacted and died, 18 did not react.

   Of these 18 non-reacters, 11 were tested later by subcutaneous injections of 1 and 2 c.c. (same strain), all reacted and 5 died. One was tested with 10 c.c. and reacted and died; 6 were tested with 3 c.c. (different strain), all reacted and died.

2. In the dose of 3 c.c. subcutaneously, 3 reacted and recovered. 2 reacted and died, 2 did not react.

   When tested later by subcutaneous injection of 2 c.c. (different strain), both reacted and died.

3. In the dose of 5 c.c. subcutaneously, 2 reacted and recovered, 2 reacted and died, 5 did not react.

   Of these 5 non-reacters, 4 were tested subcutaneously with 2 c.c. (different strain), all reacted and died; and 1 was tested with 2 c.c. (same strain) and reacted and died.

4. In the dose of 10 c.c. subcutaneously, 5 did not react.

   Four were tested later with the same strain and reacted and recovered; 1 was tested with a different strain and reacted and recovered (doses 1, 2, and 3 c.c.).

5. In the dose of 20 c.c. subcutaneously, 1 reacted and recovered.

6. In the dose of 50 c.c. subcutaneously, 1 did not react.

   When tested later by subcutaneous injection of 2 c.c. (same strain) this animal reacted and died.

7. In the dose of 2 c.c. intrajugularly, 2 reacted and died.

8. In the dose of 5 c.c. intrajugularly, 2 reacted and died.

9. In the dose of 10 c.c. intrajugularly, 1 reacted and recovered.

EXPERIMENT

With Mule Virus, Type 2732,

HORSE 1087

I. Generation,
II Generation,
III Generation,
IV Generation.

Gen.	Date of Injection.	Species.	No.	Virus. Inj.	Orig.	Gen.	Date.	Reaction from.	Incub.
	1907.						1907.	1907.	(days)
V	April 2	Mule	2732	S. 2	2415	4	Jan. 2	April 8 to 16	6
	1908.							1908.	
VI	Feb. 26	,,	3286	S. 2	2732	5	April 12	—	—
	Mar. 20	Horse	3431	,,	,,	,,	,, 12	March 25 to 30	5
	Aug. 17	,,	2838	I. 5	,,	,,	,, 11	Aug. 24 to 28	7
	April 6	Mule	3398	S. 2	,,	,,	,, 12	April 12 to 17	6
	1907.								
	July 27	,,	2894	S. 2	2732	5	,, 11	—	2894 pr not vi
VII	Aug. 11	,,	2957	S. 5	2894	6	Aug. 11	No reaction	—
	1908.						1908.		
	April 20	,,	3361	S. 2	3398	6	April 14	April 25 to May 3	5
	,, 20	Horse	3451	,,	,,	,,	,, 14	April 26 to May 5	6
	May 12	,,	3306	,,	,,	,,	,, 14	May 19 to 26	7
	,, 29	,,	3252	,,	,,	,,	,, 14	June 3 to 8	5
	July 21	,,	3356	,,	,,	,,	,, 14	July 28 to Aug. 5	7
VIII	May 12	,,	3524	,,	3451	7	,, 29	May 17 to 22	5
							1907.		
VI	Oct. 8	Mule	3900	,,	2732	5	April 12	Oct. 12 to 24	3
	,, 8	,,	3954	,,	,,	,,	,, 12	,, 15 to 24	6
	,, 8	,,	3972	,,	,,	,,	,, 12	,, 14 to 24	5

No. 6.

Tzaneen strain, fifth generation.

(Origin).

Mule 1965.
Mule 1996.
Mules, Natal.
Mule 2415.

Reaction.	Incub. + R.	Result.		Tested.	Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1907.						
8	14	R & D	1	April 23	S. 2	2148	Tzn.	—	No R	
			2	May 7	S. 6	2709	Ord.	—	,,	
						2148	Tzn.	—	,,	
						2168	Bul.	—	,,	
—	—	R	–	—	—	—	—	—	—	Died 12/4/08 of pneumonia interstitial.
5	10	R D †								
4	11	R †								
				1908.						
5	11	Recovd.	1	April 20	S. 2	2891	Tzn.	4	,,	
			2	May 7	,,	3494	P.P.R.	1	,,	
					,,	3501	CD VI	1	,,	
					,,	3272	CD V	1	,,	
				1907.						
obably rulent	—	Indef. R	–	Aug. 22	S. 2	2884	CD	3	,,	Simult. 150 c.c. serum.
				Oct. 4	I. 5	2172	Bul.	—	Slight R	
—	—	R ?	–	Nov. 21	,,	3126	CD Mixt.	—	R D †	
				1908.						
8	13	Recovd.	1	May 7	S. 2	2891	Tzn.	4	No R	
			2	June 2	,,	2884	CD	3	Slight R	Simult. 100 c.c. serum.
					,,	3272	CD V	1	,,	,, ,,
					,,	2501	CD VI	1	,,	,, ,,
					,,	3494	P.P.R.	1	,,	,, ,,
9	15	,,	–	May 23	T. 8500	3544	Simp. III	—	No R	
7	14	R & D	–	June 14	T. 10000	3608	Tzn.	19	R	
5	10	R D †								
8	15	Recovd.	–	Aug. 14	S. 2	3749	,,	21	No R	After 48 hrs. 200 c.c. serum.
				Sept. 2	T. 10000	3816	,,	24	,,	
5	10	R †								
13	16	Recovd.	–	Oct. 29	S. 3	—	Polyv. VI	—	,,	Simult. 100 c.c. serum.
10	16	,,	–	,, 29	,,	—	,,	—	,,	,, ,,
11	16	R & D	–	,, 29	,,	—	,,	—	,,	,, ,,

R—Reaction. RD—Reaction with dikkop. R?—Doubtful reaction. RD†—Reaction with dikkop and died. R†—Reaction and died.

EXPERIMENT

TYPE

IV Generation.
V Generation,
VI Generation,
VII Generation,

Gen.	Date of Injection.	Species.	No.	Virus.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.	1908.	(days)
VIII	May 7	Mule	3433	S. 5	3361	7	April 29	May 15 to 23	8
	,, 7	,,	3509	I. 5	,,	,,	,, 29	,, 11 to 20	4
	,, 7	Donkey foal	3205	,,	,,	,,	,, 29	,, 12 to 21	5
	,, 14	Horse	3424	S. 2	,,	,,	,, 29	May 22 to June 1	8
	,, 21	,,	3535	,,	,,	,,	,, 29	May 30 to June 6	9
	,, 29	,,	3571	,,	,,	,,	,, 29	June 6 to 13	8
	June 3	Mule	3389	,,	,,	,,	,, 29	,, 10 to 17	7
	,, 3	Horse	3480	,,	,,	,,	,, 29	,, 14 to 22	11
	,, 3	,,	3492	,,	,,	,,	,, 29	,, 12 to 15	9
	,, 5	,,	3547	,,	,,	,,	,, 29	,, 11 to 16	6
	,, 5	,,	3553	,,	,,	,,	,, 29	,, 14 to 23	9
	,, 26	,,	3579	,,	,,	,,	,, 29	—	—
	,, 30	Mule	3529	,,	,,	,,	,, 29	July 4 to 11	4
	July 4	Horse	3625	,,	,,	,,	,, 29	,, 8 to 11	4
	,, 4	,,	3629	,,	,,	,,	,, 29	,, 8 to 11	4
IX	May 21	,,	3555	,,	3509	8	May 20	May 31 to June 4	10
	June 3	Mule	3559	,,	3535	,,	June 1	—	—

No. 6—(*continued*).

2732.

Mule 2415.
Mule 2732.
Mule 3398.
Mule 3361.

Reaction.	Incub. + R.	Result.		Tested.	Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
7	15	Recovd.	–	June 2	S. 8	2884	CD	3	R	Simult. 100 c.c. serum.
						3272	CD V	1	,,	
						3501	CD VI	1	,,	
						3494	P.P.R.	1	,,	
9	13	Recovd.	–	,, 2	,,	2884	CD	3	Indef. R	
						3272	CD V	1	,,	
9	14	,,		—	—	3501	CD VI	1	,,	
9	17	R D †	–			3494	P.P.R.	1	,,	
7	16	,,				—	—	—	—	
7	15	R & D	1	June 30	S. 2	3619	Tzn.	20	R & D	
			2	July 23	T. 10000	3749	,,	21	Slight R	
7	14	Recovd.	–	June 26	S. 3	—	Polyv. VI	—	No R	
8	19	R D †								
3	12	R †								
6	12	R †								
9	18	Recovd.	–	,, 30	S. 2	3619	Tzn.	20	R	Died 20/7/08 of pneumonia.
—	—	Indef. R	–	July 29	,,	3749	,,	21	R †	After 48 hrs. 200 c.c. serum.
7	11	R †								
3	7	,,								
3	7	,,								
4	14	,,								
—	—	Irreg. R D and *Piro. equi*	–	June 26	S. 3	—	Polyv. VI	—	R	

R—Reaction. R & D—Reaction with dikkop. R†—Reaction and died.
RD†—Reaction with dikkop and died.

EXPERIMENT

TYPE

VII Generation,
VIII Generation.
VIII Generation,

Gen.	Date of Injection.	Species.	No.	VIRUS.				Reaction from.	Incub.
				Inj.	Orig.	Gen.	Date.		
	1908.						1908.	1908.	(days)
IX	May 21	Mule	3529	S. 2	3205	8	May 19	—	—
	June 9			I. 2	,,	,,	,, 19	—	—
	May 21	Horse	3547	S. 2	,,	,,	,, 19	—	—
	June 3	,,	3588	,,	,,	,,	,, 19	—	—
	,, 3	,,	3613	I. 2	,,	,,	,, 19	—	—
	,, 11	,,	3620	I. 10	,,	,,	,, 19	June 18 to 23	7
	,, 23	Mule	3652	,,	,,	,,	,, 19	—	—
	July 4	Horse	3604	S. 2	,,	,,	,, 19	—	—
	,, 21			I. 2	,,	,,	,, 19	—	—
	May 21	Horse	3549	S. 2	3433	,,	,, 18	May 29 to June 3	8
	,, 21	Mule	3508	,,	,,	,,	,, 18	—	—
	June 11			I. 2	,,	,,	,, 18	—	—
	,, 3	Horse	3608	S. 2	,,	,,	,, 18	June 14 to 21	11
	,, 3	Mule	3531	,,	,,	,,	,, 18	,, 10 to 21	7
	,, 4	Donkey foal	3575	I. 10	,,	,,	,, 18	—	—
	July 4	Horse	3632	S. 2	,,	,,	,, 18	—	—
X	,, 21			I. 2	,,	,,	,, 18	June 26 to July 3	5
	June 23	Mule	3651	S. 2	3620	9	June 22	June 27 to July 8	4

No. 6—(*continued*).

2732.

Mule 3361.
Donkey foal 3205.
Mule 3433.

Reaction.	Incub. + R.	Result.	Tested.		Virus. Inj.	Orig.	Qual.	Gen.	Result.	Remarks.
(days)	(days)			1908.						
—	—	No R	–	—	—	—	—	—	—	
—	—	No R	–	June 30	S. 2	3361	Tzn.	7	R †	
—	—	Indef. R	–	„ 5	„	„	„	„	„	
—	—	No R	1	„ 19	I. 20	3508	„	9	No R	
			2	July 4	S. 2	3398	„	6	R & D	
			3	Sept. 2	T. 10000	3841	„	24	R & *Piro equi*	
—	—	Indef. R	1	June 19	S.10	3574	„	7	No R	
			2	July 21	I. 2	„	„	„	„	
			3	Aug. 14	I.10	3737	„	8	Slight R	
			4	Sept. 9	S. 3	—	Polyv. VI	—	No R	Simult. 300 c.c. serum.
5	12	R D †								
—	—	No R	–	July 31	S. 2	3699	Tzn.	6	R †	
—	—	„	–	—	—	—	—	—	—	
—	—	„	1	Aug. 11	S. 1	3557	Tzn.	8	R ?	Simult. 300 c.c. serum.
						3270	„	7	„	
			2	„ 27	S. 3	—	Polyv. VI	—	Slight R	„ „
			3	Sept. 17	T. 10000	3888	CD VI	—	No R	
5	13	R D †								
—	—	Indef. R	–	—	—	—	—	—	—	
—	—	No R	–	June 6	S. 3	—	Polyv. VI	—	R	
7	18	R D †								
11	18	„								
—	—	Indef. R	–	—	—	—	—	—	—	
—	—	No R	–	—	—	—	—	—	—	
7	12	R †								
11	15	Recovd.	–	July 21	S. 3	—	Polyv. VI	—	Slight R	After 3 days 100 c.c. pol. serum

R—Reaction. R & D—Reaction with dikkop. R?—Doubtful reaction.
RD†—Reaction with dikkop and died. R†—Reaction and died.

Analysis of Results—Type 2732.

"A." *Susceptibility.*

Results.

1. Of 23 subcutaneous injections into susceptible horses, 5 reacted and recovered, 13 reacted and died, 5 did not react.
2. Of 5 intrajugular injections into susceptible horses, 3 reacted and died, 2 did not react.
3. Of 17 subcutaneous injections into susceptible mules, 10 reacted and recovered, 2 reacted and died, 5 did not react.
4. Of 4 intrajugular injections into susceptible mules, 1 reacted and recovered, 3 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
Subcutaneous injections into horses caused	78	56	22
,, ,, ,, mules ,,	70	10	30
Intrajugular ,, ,, horses ,,	60	60	40
,, ,, ,, mules ,,	25	Nil.	75

Conclusions.

1. Horses were more susceptible than mules.
2. The susceptibility of horses is greater than that of mules in a subcutaneous injection.

"B." *Resistance.*

Results.

1. One horse injected subcutaneously with 2 c.c. virus did not react, but died when injected with the same virus intrajugularly.
2. One mule injected subcutaneously and intrajugularly in the dose of 2 c.c. did not react, but died when injected with virus of the same type, previous generation.
3. One horse injected subcutaneously and intrajugularly in the dose of 2 c.c. did not react, but when tested with different type gave a doubtful reaction, and on a succeeding test with a different strain a slight reaction resulted.
4. One mule injected subcutaneously and intrajugularly in the dose of 2 c.c. virus did not react, but showed a reaction when tested with a different strain.

"C." *Virulency.*

Results.

1. One susceptible horse injected with horse virus reacted and died.
2. Of 2 susceptible mules injected with horse virus, 1 reacted and recovered, 1 did not react.
3. Of 21 susceptible horses injected with mule virus, 5 reacted and recovered, 14 reacted and died, 2 did not react.
4. Of 14 susceptible mules injected with mule virus, 10 reacted and recovered, 2 reacted and died, 2 did not react.
5. Of 5 susceptible horses injected with donkey foal virus, 1 reacted and died, 4 did not react.
6. Of 2 susceptible mules injected with donkey foal virus, 2 did not react.

Taking these figures out according to percentages,

	Reactions.	Deaths.	No Reaction.
	%	%	%
The injection of horses with horse virus caused	—	—	—
,, ,, mules ,, ,, ,, ,,	—	—	—
,, ,, horses ,, mule ,, ,,	90	65	10
,, ,, mules ,, ,, ,, ,,	85	15	15
,, ,, horses ,, donkey foal virus caused	20	20	80

Conclusions.

1. Horses were more susceptible to the injection of mule virus than mules.
2. The injection of mule virus into horses caused the greatest mortality.
3. A very high percentage of animals remained refractory to the injection of donkey foal virus.

"D." Variability.

5th Gen. ... ... M. 2732—100 % R. (8 animals).

6th Gen. ... ... M. 3298—100 % R. (5 animals).

7th Gen. ... ... M. 3361—93 % R. (14 animals).

8th Gen. ... ... D.F. 3205—12 % R. (9 animals). M. 3433—80 % R. (5 animals).

Conclusions.

1. In the case of this type the generation seems to affect the virus, but, notwithstanding this, the virulency remains high.
2. Donkey foal virus after passing through a donkey has decreased enormously in virulency.

"E." Influence of dose and manner of injection.

Injection of virus of type '2732.

1. In the dose of 2 c.c. subcutaneously, 13 reacted and recovered, 16 reacted and died, 8 did not react.
   Of these 8 non-reacters, 4 were tested later by a subcutaneous injection of 2 c.c., 3 reacted and died, 1 reacted and recovered.
   One was injected with 2 c.c. intrajugularly and reacted and died, 3 were injected with 3 c.c. subcutaneously, all 3 reacted and recovered.
2. In the dose of 5 c.c. subcutaneously, 1 reacted and recovered, 1 did not react.
   When tested later by intrajugular injection of 5 c.c., this animal reacted and died.
3. In the dose of 2 c.c. intrajugularly, 1 did not react.
   When tested later by intrajugular injection of 10 c.c.. this animal reacted and recovered.
4. In the dose of 5 c.c. intrajugularly, 1 reacted and recovered, 1 reacted and died.
5. In the dose of 10 c.c. intrajugularly, 1 reacted and recovered, 1 did not react.
   When tested later by subcutaneous injection of 2 c.c. this animal, reacted and died.

Summary of Results given by Various Types arranged according to Dose.

Percentage of Reactions caused by injection of Virus in Varying Doses (as per Col. 1).

	Type 2891.	Type 2694.	Type 2415.	Type 2732.	Type 2539.	Type 1965.
	%	%	%	%	%	%
Subcutaneous injections of 2 c.c.	10	62	11	22	55	31
,, ,, ,, 3 c.c.	—	30	25	—	—	—
,, ,, ,, 5 c.c.	—	55	—	—	—	—
,, ,, ,, 10 c.c.	—	100	—	—	—	—
,, ,, ,, 20 c.c.	—	—	—	—	—	—
,, ,, ,, 50 c.c.	—	—	—	—	—	—
Intrajugular injections of 2 c.c.	—	—	—	—	75	—
,, ,, ,, 3 c.c.	—	—	—	—	—	—
,, ,, ,, 5 c.c.	—	—	—	—	—	25
,, ,, ,, 10 c.c.	—	—	—	—	—	33
,, ,, ,, 50 c.c.	—	—	—	—	—	—
Total	15	54	14	25	55	32

1. Arranged according to virulency, the various types show the following ascending order :—2415, 2891, 2732, 1965, 2694, and 2539.

2. The differences in the virulency is particularly marked ; 2891 and 2415 correspond, and these two vira both originated from the Natal virus. Again with types 2539, 2694, and 2732, these all directly descended from type 2415, yet 2732 only causes 25 per cent. reactions, whereas 2539 and 2694 caused 55 per cent. and 54 per cent. respectively.

Conclusions.

1. The virus Tzaneen has in no instance been virulent for all mules injected ; this virulency differs, so to say, from animal to animal ; one particular animal seems to influence the virus in such a way that this virulency is either decreased or increased.

2. This reduction of virulency is by no means the result of the dose of virus or of method of injection ; small and large doses, injected subcutaneously or intrajugularly, equally fail to produce reactions.

3. This difference in virulency is either due to the virus itself or to the injected animal, but considering that animals which resisted quantities of virus of one particular type, subcutaneously or intrajugularly, contract horse-sickness from a subsequent subcutaneous injection of a smaller quantity, it shows that it is not so much the resistance of the animal but the virulency of the virus of the given animal.

4. It is probably correct to conclude that both animal and virus must be in a certain relation to each other before a reaction can ensue.

G.—FEVER REACTIONS IN HORSES SIMULATING HORSE-SICKNESS.

In a publication of mine, which appeared in the " Deutsche Wochenschrift Thiermedicine " in 1907, I described a fever reaction in horses under the title of " Ephemeral fever in horses," resembling horse-sickness in character, but of a shorter duration and which did not end fatally.

It was proved at that time that a recovery from this ephemeral fever did not produce any immunity against horse-sickness. The fact that microscopical examination of blood taken from animals suffering from either horse-sickness or ephemeral fever gave negative results and that the virus of both fevers could be preserved for any length of time, suggested some connection between the two, although the immunity not being reciprocal, negatived the identification for the time being.

During the experiments on horse-sickness a similar occurrence was noted again, and this was followed up for some time on numerous animals, the object being again to note whether the reaction had any connection with the disease.

At the same time a number of experiments were made by exposing horses during the night, when it was noticed that one of them showed a reaction, again typical for horse-sickness. The blood of this animal was also used for inoculation purposes, but did not create immunity; however, since the fact was noted that even virulent strains of horse-sickness virus did not give absolute immunity against each other, it was considered advisable to proceed further into the question, accepting the possibility that there may yet be a connection between the two.

Experiment No. 1.

With an ephemeral fever virus known as the "Two-mule Tzaneen Virus."

1. Origin of this strain.

Mule 1995.—Injected on 4th August, 1909, with virus 1965, originating from horse 1087, known as the origin of virus Tzaneen.

Result.—Contracted horse-sickness and died.

Mule 1996.—Injected as above.

Result.—Contracted horse-sickness and recovered.

The reaction of 1996 was very mild, lasting from 4th to 9th day, almost atypical.

This animal was tapped on the 7th day after injection, and this blood was virulent, in as much as it produced horse-sickness in the mule 2034, which on 11th August was injected intrajugularly with 5 c.c. virus 1996 of the same date, and mule 2034 died of horse-sickness nine days after injection.

Virus 1996 was utilised for immunisation in practice when it was noticed that of the animals injected, not all of them reacted with a simultaneous injection of serum; this was more particularly the case in Natal.

2. First generation.

Mule 2691.—Injected on 12th March, 1907, with 2 c.c. intrajugularly with virus mule 1996 (returned from Natal); and

Mule 2692.—Injected on 12th March, 1907, with 10 c.c. intrajugularly with virus mule 1996 (returned from Natal).

Results.—In both instances an irregular reaction followed, but not typical for horse-sickness. During these reactions the animals were tapped on several occasions. They were then submitted to a test with virus mule 2415, Tzaneen, fourth generation, when both contracted typical horse-sickness reactions and recovered; 2692 showing also dikkop.

With the blood collected from mule 2691 on the 24th March (twelve days after injection) and from 2692 on the 26th March (fourteen days after injection), a mixture of equal quantities was made.

Mule 3285.—Injected on 26th February, 1908, with 4 c.c. subcutaneously of this mixture.

Result.—Reaction followed from the 6th to the 9th day, reaching 104 on the 6th day and 103·6 on the two following days. The animal was tapped on the 8th day, *and the virus called the "Two-mule Tzaneen Virus."*

Tested on the 20th April, 1908, with 2 c.c. virus mule 2415, Tzaneen, fourth generation.

Result.—Slight reaction with dikkop on 11th day, and recovered.

"*A.*" *Injections with mule virus* 3285, *dated 5th March*, 1908 (*Two-mule Tzaneen virus*).

3. Second generation.

"A." *Horse* 3404.—Injected on the 10th March, 1908, with 2 c.c. subcutaneously with virus mule 3285.

Result.—Reaction from the 6th to the 10th day; reaching 106 in the evening of the 8th day. Pulse averaged 46 during this reaction.

Horse 3404 was in an experiment later with virus 3265 (exposure)* on the 30th March, 1908, and on 15th April with virus 3266 (four-mule† Tzaneen virus). Finally on the 27th April it was injected simultaneously and subcutaneously with serum and virus 2415, when it contracted horse-sickness and died.

"B." *Mule* 3279.—Injected on the 10th of March subcutaneously with 2 c.c. virus.

Result.—Reaction from the 3rd to the 10th day, reaching 104·6 on 5th and 6th day, when the animal was tapped (16th and 17th March). On 30th March injected with virus 3265 (exposure virus), and 11th April with virus 3266 (four-mule Tzaneen virus).

Tested on 20th April with 2 c.c. virus mule 2415, Tzaneen, fourth generation.

Result.—Reaction again followed from the 6th to the 11th day.

"C." *Horse* 3331.—This animal was previously used on 10th March for virus 3390 (four-mule Tzaneen virus) without reaction. Injected subcutaneously on the 30th March, 1908, with 2 c.c. virus mule 3285.

Result.—No reaction.

Injected on the 15th April with virus 3261 (exposure). Finally submitted to simultaneous injection of serum and virus 2415.

Result.—Contracted horse-sickness and died.

"D." *Horse* 3250.—Injected subcutaneously on the 30th March, 1908, with a mixture of 10 c.c. virus mule 3285, and 100 c.c. serum CD.

Result.—Reaction from 5th to 9th day, reaching 106 on evening of 7th day. The animal was bled on the 8th day.

Injected on the 15th April with virus 3266 (four-mule Tzaneen virus).

Result.—No reaction.

Injected on the 7th May subcutaneously with virus 2926 (donkey foal), Simpson III.

Result.—No reaction.

Injected on the 21st May with 2 c.c. same virus, intrajugularly.

Result.—No reaction.

Injected on 3rd June with 2 c.c. same virus (2926), intrajugularly.

Result.—Horse-sickness reaction developed, of which the animal died.

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* See experiment No. 4.

† See experiment No. 2.

4. Third generation.

"E." *Horse* 3328.—Injected on the 20th March, 1908, subcutaneously with 5 c.c. virus mule 3279.

Result.—Reaction from 4th to 8th day, with an evening temperature of 105·2 on 5th day and a pulse of 84 and 88 on 5th and 6th days.

Tested on the 6th April with 2 c.c. virus 2891, Tzaneen.

Result.—Developed horse-sickness with dikkop and recovered.

"F." *Horse* 3373.—Injected on the 20th March, 1908, with 5 c.c. subcutaneously virus horse 3404 (19th March, 1908).

Result.—Reaction from 4th to 8th day, with an evening temperature of 105·6 on 5th day and 105·4 on 6th day, and a pulse of 62 and 76 respectively.

Injected on the 30th March with 2 c.c. virus 2891, Tzaneen, subcutaneously.

Result.—Contracted horse-sickness and died.

"G." *Mule* 3422 (Immune to virus 2884, CD).—Injected on the 30th of March, 1908, with 2 c.c. virus horse 3404, subcutaneously. Rise of temperature from 6th to 11th days with maximum temperature of 103·2 on evening of the 7th day.

"H." *Mule* 3423 (Immune to virus 2884, CD).—Injected as above. Sharp rise from 5th to 10th days; highest temperature 103·8 on evening of 6th day.

"I." *Horse* 3239.—Injected on the 30th March, 1908, subcutaneously with 10 c.c. virus horse 3404, and 100 c.c. serum C.D.

Result.—Reaction from 4th to 10th days, highest temperature 105·6 on evening of the 6th day with a pulse of 70.

Injected on the 15th April with virus 3266 (four-mule Tzaneen virus).

Result.—No reaction.

Injected in August, 1908, simultaneously with serum and virus Tzaneen.

Result.—Reaction and recovered.

"J." *Mule* 3278.—This animal was previously injected on 10th March, 1908, with virus 3390 (four-mule Tzaneen virus).

Result.—Slight reaction.

Injected on the 6th April, 1908, with 2 c.c. virus horse 3404, subcutaneously, dated 19th March, 1908 (two-mule Tzaneen virus).

Result.—No reaction.

Tested on the 18th April with 2 c.c. virus mule Tzaneen, fourth generation.

Result.—Slight reaction.

"K." *Horse* 3464.—Infused on 6th April with 3000 c.c. virus horse 3250.

Result.—On evening of 7th day temperature noted of 103, but this was the only indication of a disturbance.

Tested on the 22nd April with virus mule 3359, Tzaneen.

Result.—Contracted horse-sickness and recovered.

5. Fourth generation.

"L." *Horse* 3252.—Injected on the 11th April subcutaneously with 2 c.c. virus horse 3239 (7th April, 1908).

Result.—Reaction from the 5th to the 15th day with an average evening temperature of 104.

Tested on the 29th of May with 2 c.c. virus mule 3398, Tzaneen.

Result.—Developed horse-sickness and died.

"M." *Horse foal* 1993 (immune to virus 2884, CD).—Injected on the 11th April subcutaneously with 2 c.c. virus horse 3239 (7th April, 1908).

Result.—Slight reaction from 5th to the 12th day; maximum temperature 102·6 on evening of the 7th and 9th days.

"N." *Horse foal* 1997 (immune to virus 2884, CD).—Injected on the 11th April as above.

Result.—Slight reaction from the 2nd to the 13th days; highest temperature 103·6 on evening of 9th day.

"O." *Horse* 3470.—Injected on the 11th April, 1908, subcutaneously with 100 c.c. virus horse 3239 (7th April, 1808).

Result.—Reaction started the following day and lasted to the 9th day; average temperature 103.

Tested in July simultaneously and subcutaneously with serum and virus 3398, Tzaneen.

Result.—Contracted horse-sickness and recovered.

Results.

Virus 1996, Tzaneen, second generation, used on the station produced horse-sickness. Virus 1996, Tzaneen, second generation, issued to Natal and subsequently returned when injected into two mules, gave atypical reaction. These two mules were tested later with Tzaneen, fourth generation virus, and both showed reactions and recovered. Blood collected at the time of the atypical reaction from these two mules was mixed, and the mixture is referred to as the two-mule Tzaneen virus. This mixture serves as the origin of the virus used.

First generation (of ephemeral fever virus).

Of 2 mules and 1 horse injected with two-mule Tzaneen virus, 1 mule and 1 horse showed reactions. When tested later with Tzaneen, fourth generation, all showed horse-sickness reactions and 1 horse and 1 mule died.

Second generation.

Of 3 mules and 4 horses injected with two-mule Tzaneen virus, first generation, 2 mules and 4 horses gave reactions.

One mule and 4 horses were tested later and all showed reactions, and 2 horses died of horse-sickness.

Third generation.

Of 3 horses and 2 horse foals injected with two-mule Tzaneen virus, second generation, 2 horses gave reactions, the 2 horse foals slight reactions and the other horse a very slight reaction. (This last animal had been infused with 3000 c.c. of the mixture, and when tested later with Tzaneen, sixth generation, showed horse-sickness reaction and recovered.) The other 2 horses were also tested with Tzaneen, sixth generation, and both showed horse-sickness reactions, 1 died.

Conclusions.

The injection of two-mule Tzaneen virus strain produced fever reactions. No mortality occurred as a result of this injection, notwithstanding the high fever and pulse.

No immunity was given by this reaction against subsequent injections of horse-sickness virus. The quantity of virus injected had no influence on the severity of the clinical symptoms.

The immunity obtained from the two-mule virus protected against subsequent inoculation of the four-mule virus (see also later).

Animals immune against horse-sickness when injected with two or four mule Tzaneen virus showed reactions.

Animals injected with a mixture of two-mule Tzaneen virus and horse-sickness serum showed reactions.

Animals which had reactions from the two-mule Tzaneen virus, contracted horse-sickness, and the majority died when injected with horse-sickness virus.

Experiment No. 2.

With an ephemeral fever virus known as the "Four-mule Tzaneen Virus.

The virus of mule 1996, which was utilised in Natal, produced a reaction in two mules, accompanied with lesions of dikkop. They were tapped and the blood forwarded to our station. Several other experiments were made, the principal animal of which was mule 2415 (compare article the "Variability of a given strain of horse-sickness virus").

The blood obtained on 2nd January, 1907, from mule 2415 was injected into mule 2539. Reaction began after three days and was over on the 11th day. It was typical in character for horse-sickness, but rather short in duration. The animal recovered (for tests, *vide* article quoted above).

Mule 2539 was tapped on the 6th and 8th days after injection. Blood of this reaction taken on the 8th day was virulent for mule 2495 (4th February, 1907) (subcutaneously 2 c.c.), and again for horse 3248 (17th February, 1908), subcutaneously 50 c.c. (*vide* article referred to). The blood of mule 2539 taken on the 6th day was used for injection of the following two animals: Mules 2693 and 2694, and blood of the 8th day for injection of mules 2669, 2695, and 2696.

"A." *Mule* 2669.—Injected on the 27th February, 1907, subcutaneously with 2 c.c. virus mule 2539, Tzaneen, fifth generation (30th January, 1907).

Result.—An indication of a reaction, but nothing definite. Animal was bled on the 11th day.

Tested on the 20th March with 5 c.c. virus mule 2415, Tzaneen, fourth generation, and had a typical horse-sickness reaction and recovered

"B." *Mule* 2693.—Injected on the 20th March subcutaneously with 2 c.c. virus mule 2539 (28th January, 1907).

Result.—No distinct reaction. Animal bled on the 8th day.

Tested on the 4th of April with virus 2629, Tzaneen, fifth generation (14th February, 1907).

Result.—Typical horse-sickness reaction resulted and recovered.

"C." *Mule* 2694.—Injected on the 20th March, 1907, subcutaneously with 2 c.c. virus mule 2539 (28th January, 1907).

Result.—No definite reaction, but some irregularity in the temperature was noted, and animal was bled on the 8th day.

Tested on the 4th April with virus mule 2415.

Result.—Developed horse-sickness reaction with dikkop on the 14th day and recovered.

"D." *Mule* 2695.—Injected on the 20th March, 1907, subcutaneously with 2 c.c. virus mule 2539 (30th January, 1907).

Result.—No definite reaction, but an irregular slight disturbance in the temperature curve. Animal was tapped on the 8th day.

Tested on the 4th April with virus 2629, Tzaneen.

Result.—Reaction and recovered.

"E." *Mule* 2696.—Injected on the 20th March, 1907, subcutaneously with 2 c.c. virus mule 2539 (30th January, 1907).

Result.—Slight irregular temperature reaction, in no way typical for horse-sickness. Animal was tapped on the 8th day.

Tested on the 4th of April with virus mule 2415, Tzaneen.

Result.—Reaction and recovered.

Experiments with a mixture of virus of mules 2669 (10th March, 1908), 2693 (28th March, 1908), 2694 (28th March, 1908), and 2695 (28th March, 1908) in equal quantities; of this mixture one mule was injected, viz.:—

"F." *Mule* 3390.—Injected on the 26th February, 1908, subcutaneously with 8 c.c. of above mixture.

Result.—Reaction from 6th to 11th days, resembling a horse-sickness reaction in character with maximum temperature of 103·6 on 8th day and a pulse record of 60 on the 8th, 9th, and 10th days. The animal was tapped on the 8th, 9th, and 11th days, and this blood (3390) is called the four-mule Tzaneen virus.

Mule 3390 was tested on the 20th of April with 2 c.c. virus mule 2415, Tzaneen, fourth generation.

Result.—Developed a typical horse-sickness reaction and recovered.

Second generation.

"G." *Horse* 3331.—Injected on the 10th of March, 1908, subcutaneously with 2 c.c. virus mule 3390 (6th March, 1908).

Result.—Irregular temperature disturbance, but not of a definite character. In an experiment with serum and virus 2415 on the 23rd of April; contracted horse-sickness and died.

"H." *Mule* 3278.—Injected on the 10th of March, 1908, subcutaneously with 2 c.c. virus mule 3390 (6th March, 1908).

Result.—Slight reaction on the 4th, 5th, and 6th days. The animal was bled on the 6th day.

Injected on the 6th of April with virus 3404, two-mule Tzaneen.

Result.—No reaction.

Tested on the 18th of April with virus mule 2415.

Result.—Slight reaction.

Third generation.

"I." *Horse* 3266.—Injected on the 30th of March subcutaneously with 2 c.c. virus mule 3278 (16th March, 1908).

Result.—Reaction from 7th to 11th days with maximum temperature of 104 on 3rd day. Bled on the 8th and 9th days.

Injected with virus 3239 (two-mule Tzaneen virus).

No result.

Finally used in an experiment with serum and virus, Tzaneen.

Result.—Contracted horse-sickness and died.

Fourth generation.

J." *Horse* 3274.—Injected on the 11th of April subcutaneously with 2 c.c. virus horse 3266 (8th April, 1908).

Result.—Reaction from 4th to 9th days with a maximum temperature of 105 and pulse 54. Bled on the 7th day.

Tested on the 7th July with virus 3406, Tzaneen, seventh generation.

Result.—Contracted horse-sickness and died.

"K." *Horse* 3460.—Injected on the 11th of April subcutaneously with 100 c.c. virus horse 3266 (8th April, 1908).

Result.—Irregular temperature from the day after injection for a week.

Injected on the 21st of July with virus 3619, Tzaneen, twentieth generation, three days later injected with serum.

Result.—Contracted horse-sickness and died.

"L." *Horse foal* 1766 (immune to virus 2884, CD).—Injected on the 11th of April subcutaneously with 2 c.c. virus horse 3266 (8th April, 1908).

Result.—Reaction from 4th to 9th days.

"M." *Horse foal* 1991 (immune to virus 2884, CD).—Injected on the 11th of April, as above.

Result.—Reaction from the 2nd to the 10th days; highest temperature of 104·2 on evening of 5th day.

Results.

The reaction obtained from an inoculation of four-mule Tzaneen virus corresponds in character to that obtained in the previous experiments with the two-mule Tzaneen virus, and since the origin of these two vira is the same, we have a reason to expect this to be the case.

Since immunity obtained from the four-mule virus protected against the inoculation of the two-mule virus (see previous experiments) further support is given to our conception.

Further since the immunity obtained from the two-mule virus protects against the four-mule virus, we have to accept that these two vira are the same.

Experiment No. 3.

With a mixture of Tzaneen two-mule virus and four-mule virus.

"A." *Horse* 3383.—Injected on the 11th of April subcutaneously with 100 c.c. of a mixture of 3266 (four-mule virus) and 3239 (two-mule virus).

Result.—Reaction from 3rd to the 10th days, with a maximum temperature of 105 on the 6th day.

Injected with virus 3749, Tzaneen, twenty-first generation, succeeded by serum.

Result.—Contracted horse-sickness and died.

"B." *Horse* 3259.—Injected on the 15th of April subcutaneously with 4 c.c. of above mixture.

Result.—Reaction on 5th, 6th, and 7th days. Maximum temperature 105 on the 6th day.

Injected on the 19th June with virus donkey foal 2926, Simpson III.

Result.—Contracted horse-sickness and died.

"C." *Horse* 3338.—Injected on the 18th of April subcutaneously with 4 c.c. of above mixture.

Result.—Slight reaction on the 10th, 11th, and 12th days.

Injected on the 7th of May simultaneously with serum and virus, Tzaneen.

Result.—Reaction and recovered.

"D." *Horse* 3485.—Injected on the 18th of April, as above.

Result.—Temperature exacerbation on the 13th and 14th days. On 7th of May in an experiment with serum and virus 3285, Tzaneen, fifth generation,

Result.—Contracted horse-sickness and died.

" E." *Horse* 3445.—Injected on the 18th of April, as above.

Result.—No reaction; one temperature exacerbation on 13th day. In experiment on 15th June with serum and virus 3398, Tzaneen.

Result.—Contracted horse-sickness and died.

" F." *Horse* 3302.—Injected on the 18th of April, as above.

Result.—Irregular temperature with exacerbations on 8th, 9th, 12th, and 13th days.

Injected on the 29th of May with virus 3361, Tzaneen, succeeded by serum.

Result.—Reaction and recovered.

" G." *Horse* 3482.—Injected on the 18th of April, as above.

Result.—Reaction started immediately with maximum temperature of 104 on the 4th day, lasting to the 8th day. Maximum pulse 100 on 5th day, 74 on 6th day, and 64 on the 7th day.

Tested on the 7th of May simultaneously with serum and virus 3500, Tzaneen.

Result.—Contracted horse-sickness and died.

Results.

Of eight horses injected with 100 c.c. of a mixture of two and four mule Tzaneen vira, indications of reactions were noted in seven cases.

When tested later with Tzaneen virus of a higher generation of a virulent horse-sickness strain, all contracted horse-sickness and six died.

Experiment No. 4.

With an ephemeral virus, known as " Exposure Virus."

(Experiments with a horse which was exposed to natural infection of horse-sickness by letting it run during the night in a paddock at the Laboratory, Daspoort, just outside Pretoria.)

" A." *Horse* 3298 was exposed on the 15th of February in this way. This horse showed a temperature reaction resembling horse-sickness from the 5th to the 14th days, with a maximum temperature of 104·8 on the 9th day. Maximum record of pulse was 62 on the 9th day.

A microscopical examination on 9th and 12th days showed *Piroplasma equi* very rare. Examination on the 11th day gave negative results.

3298 was bled on 9th, 10th, 11th, and 12th days, and blood was used for experiments, enumerated later.

Tested on the 6th of April subcutaneously with 2 c.c. virus mule 2891, Tzaneen, fourth generation.

Result.—Contracted horse-sickness and died.

First generation.

" B." *Horse* 3265.—Injected on the 2nd of March, 1908, subcutaneously with 2 c.c. virus horse 3298 (26th February, 1908).

Result.—Reaction from 5th to 12th day with a maximum temperature of 105 on the 6th day, and a maximum pulse of 72 on the 7th day. Animal was tapped on the 6th, 8th, and 9th days.

Injected on the 15th of April with virus 3266, Tzaneen, four-mule.

Result.—No reaction.

Tested on the 4th of July with virus mule 3650, Tzaneen.

Result.—Contracted horse-sickness and died.

"C." *Horse* 3474.—Injected on the 6th of April, 1908, subcutaneously with 2 c.c. virus horse 3298 (26th February, 1908).

Result.—Hardly any reaction, only one exacerbation to 103 on the 7th day. Animal proved to be immune against horse-sickness in later experiments.

Second generation.

"D." *Horse* 3254.—Injected on the 20th of March subcutaneously with 2 c.c. virus horse 3265 (10th March, 1908).

Result.—Reaction from 6th to 10th days, with temperature disturbance up to the 14th day. Maximum temperature 106 on 7th day, and maximum pulse 54 on the 8th day.

Tested on the 6th of April with virus 3263, Simpson, third generation.

Result.—Contracted horse-sickness and recovered.

"E." *Horse* 3260.—Injected on the 20th of March subcutaneously with 10 c.c. virus horse 3265 (10th March, 1908).

Result.—Reaction from 5th to 10th days with a maximum temperature of 106 on the 7th day, and maximum pulse of 60 on same day.

Tested on the 6th of April with virus 3244, Simpson, fourth generation.

Result.—Contracted horse-sickness and died.

"F." *Horse* 3261.—Injected on the 20th of March intrajugularly with 2 c.c. virus horse 3265 (10th March, 1908).

Result.—Reaction from 5th to the 12th days with a maximum temperature of 105·8 on 7th day and maximum pulse 62 on 8th and 9th days. Animal was bled on the 6th and 7th days.

Injected on the 15th of April with 4 c.c. mixture of two and four mule virus.

Result.—Reaction from 5th to 9th days, with a maximum temperature of 104 on 6th and 7th days.

"G." *Mule* 3420 (immune to virus 2884, CD).—Injected on the 30th of March subcutaneously with 2 c.c. exposure virus horse 3265 (10th March, 1908).

Result.—Sharp rise from 7th to 11th days, with a maximum temperature of 102 on evening of the 9th day.

"H." *Mule* 3421 (immune to virus 2884, CD).—Injected on the 30th March, as above.

Result.—Rise of temperature from the 6th to the 12th days, with temperature of 102 on evening of the 7th and 9th days.

"I." *Horse foal* 2054 (immune to virus 2884, CD).—Injected on the 11th of April subcutaneously with 2 c.c. virus horse 3261 (27th March, 1908).

Result.—Irregular reaction from the 3rd to 12th days, highest temperature of 104 on evening of the 6th day.

"J." *Horse foal* 2314 (immune to virus 2884, CD).—Injected on the 11th of April, as above.

Result.—Irregular, slight reaction.

Results.—Animals immune against horse-sickness showed reactions when injected with ephemeral fever virus.

"K." *Horse* 3259.—Injected on the 30th of March subcutaneously with 2 c.c. virus, horse 3265.

Result.—No reaction.

Injected on the 15th of April with a mixture of two and four mule virus.

Result.—Reaction from the 5th, 6th, and 7th days, with a maximum temperature of 105. Died later of horse-sickness.

" L." *Horse* 3459.—Injected on the 31st of March with a mixture of 200 c.c. serum CD and 10 c.c. virus 3265.

Result.—Reaction from 5th to 10th days, with a maximum temperature of 103·4 on the 8th day.

Injected on the 21st of May simultaneously and subcutaneously with serum and virus 3398, Tzaneen.

Result.—Contracted horse-sickness and died.

" M." *Horse* 3485.—Injected on the 6th of April subcutaneously with 2 c.c. virus 3265.

Result.—Reaction from 5th to 10th days, with a maximum temperature of 106 on the 7th day.

Injected on the 18th of April with two and four mule virus.

Result.—No reaction.

Injected on the 7th of May with serum and virus 3285, Tzaneen.

Result.—Contracted horse-sickness and died.

Third generation.

" N." *Horse* 3302.—Injected on the 6th of April subcutaneously with 100 c.c. virus horse 3261 (27th March, 1908).

Result.—Reaction from 4th to 9th days, with a maximum temperature of 105·2 on 5th day.

Injected on the 18th of April with two and four mule virus (*vide* Experiment No. 3, " F ").

Result.—Irregular temperature followed.

Injected later with horse-sickness virus and recovered.

" O." *Horse* 3338.—Injected on the 6th of April intrajugularly with 100 c.c. virus 3261 (27th March, 1908).

Result.—Reaction from 2nd to 9th days, with a maximum temperature of 104·6 on 5th day.

Injected on the 18th of April with two and four mule virus.

Result.—Slight reaction.

Injected later with horse-sickness virus and recovered.

" P." *Horse* 3445.—Injected on the 6th of April subcutaneously with 2 c.c. virus 3261 (27th March, 1908).

Result.—Reaction from 4th to the 10th days.

Injected on the 18th of April with two and four mule virus.

Result.—No reaction. Later, recovered from a horse-sickness experiment.

" Q." *Horse* 3482.—Injected on the 6th of April intrajugularly with 2 c.c. virus 3261 (27th March, 1908).

Result.—No reaction.

Injected on the 18th of April with two and four mule virus.

Result.—Reaction.

" R." *Horse* 3331.—Injected previously with mule 3390, Tzaneen, four-mule virus.

Result.—No definite reaction.

Injected on the 15th of April subcutaneously with 2 c.c. virus 3261 (27th March, 1908).

Result.—No reaction. Died later of horse-sickness.

Results.—Animals injected with a mixture of this ephemeral fever virus and horse-sickness serum showed reactions.

The ephemeral fever virus did not completely protect against a later injection. Of two and four mule virus, some reactions were noted.

The immunisation of animals with exposure virus and two and four mule virus did not protect against horse-sickness.

Experiment No. 5.

With exposure virus and two-mule Tzaneen virus.

"A." *Horse* 3441.—Injected on the 6th of April subcutaneously with a mixture of 4 c.c. virus 3265 and 3404.

Result.—Reaction from 4th to the 11th days, with a maximum temperature of 106 on 6th day, and pulse record of 54.

Tested on the 20th of April simultaneously with serum and virus mule 2891, Tzaneen, fourth generation.

Result.—Contracted horse-sickness and died.

Experiment No. 6.

With exposure virus, two-mule and four-mule Tzaneen virus.

"A." *Horse* 3334 (previously injected with CD virus).—Injected on the 15th of April intrajugularly with 6 c.c. mixture of 3266 (four-mule virus), 3239 (two-mule virus), and 3261 (exposure).

Result.—No reaction. Proved to be immune against horse-sickness in subsequent experiment.

"B." *Horse* 3400.—Injected on the 15th of April, as above.

Result.—Reaction from the 2nd to the 10th days, maximum temperature 105·8 to 106 from 4th to the 8th days, and maximum pulse of 66 on the 8th day.

Injected on the 15th of June with serum and virus 3398, Tzaneen.

Result.—Reaction and recovered.

"C." *Horse* 3468.—Injected on the 15th of April, as above.

Result.—Hardly any reaction. Killed later.

"D." *Horse* 3471.—Injected on the 15th of April, as above.

Result.—Reaction from 4th to the 10th days. On the 27th of April in an experiment with serum and virus 2415, Tzaneen, reaction and recovered.

"E." *Horse* 3479.—Injected on the 15th of April, as above.

Result.—Reaction from 4th to the 7th days, with a maximum temperature of 103·8 on the 4th day.

Injected on the 14th of August with 3 c.c. virus 3659, succeeded by serum.

Result.—Contracted horse-sickness and died.

"F." *Horse* 3481.—Injected on the 15th of April, as above.

Result.—Slight temperature disturbance, with a maximum temperature of 102·4 on the 6th day.

Injected on the 21st May simultaneously with serum and virus 3263, Simpson, third generation.

Result.—Contracted horse-sickness and died.

Results.—The inoculation with a mixture of exposure and two-mule Tzaneen virus did not protect against subsequent injections of horse-sickness virus.

Of six animals injected with a mixture of (*a*) exposure, (*b*) two-mule, and (*c*) four-mule virus, two died and one was immune.

A combination of these vira did not protect against horse-sickness virus.

Summary of Conclusions.

The injection of two-mule Tzaneen virus protected against four-mule Tzaneen virus, but not completely against the exposure virus.

The two-mule Tzaneen virus, the four-mule Tzaneen virus, and the exposure virus when injected separately or as a mixture produced reactions and an acceleration of the pulse, and no animals died.

Since the two and four mule virus did not completely protect against the ephemeral fever virus, we have to conclude that these, although distinct, are to a certain extent related.

Neither of the three ephemeral fever vira protected against a horse-sickness vira, and therefore it has to be concluded that there is no connection between these ephemeral fevers and horse-sickness.

PART II.

PHYSICAL—CHEMICAL INVESTIGATIONS INTO SOUTH AFRICAN DISEASES.

By Dr. Walter Frei,
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A.—VOLUME OF BLOOD CORPUSCLES.

The percentage of the volume of blood corpuscles in comparison to the total volume of blood is found by centrifugalisation of a given volume of fresh blood until the level of the deposit remains constant. With our centrifuge and a speed of 4000-5000 revolutions pro minute this is the case after five minutes. The average error of this method amounts to about 0·3 volume per cent. absolute, or 1 per cent. in 100. Therefore the exactitude reached is greater than when counting the number of blood corpuscles, and, besides that, only a quarter of the time is needed.

The following table shows the average values of the volumes of blood corpuscles of horses in various conditions, donkeys and sheep, and the respective variations :—

Percentage of Volume of Red Blood Corpuscles.

	Normal Donkeys.	Normal Sheep.	Horses.			
			Normal.	Horse-sickness.	Immune and Hyper-immune.	Piro-plasmosis.
Number of examinations	18	8	100	100	40	30
„ animals	8	8	100	72	37	15
Average	*32·8*	*40·4*	*33·4*	*34*	*32·1*	*27·6*
„ for normal animals	—	—	33·4	33·4	33·4	33·4
Difference from average for normal animals	—	—	0	+1·8 %	—3·9 %	—17·5 %
Maximum	41	43	43	58	42	34
Minimum	20·5 %	36 %	22 %	23 %	23 %	20 %
Variation above average	25	65	29	70·5	31	23
„ below „	37·5	11	34	32·5	28	27·5
„ total	62·5	17·5	63	103	59	50·5
„ above normal average	—	—	29	74	26	1·8
„ below „ „	—	—	34	31	31	40
Values above average	56	37	48	46	48	57
„ below „	44	63	52	54	52	43
„ above normal average	—	—	48	49	45	7
„ below „ „	—	—	52	51	55	93

B.—VISCOSITY.

Viscosity and its Variations.

	Normal Horses.				Horses Suffering from Horse-sickness.		Horses Immune against Horse-sickness.			Normal Donkeys.			Normal Cattle.	Normal Sheep.		Normal Goats.
	Blood.	Defibrinated Blood.	Plasma.	Serum.	Blood.	Serum.	Blood.	Plasma.	Serum.	Blood.	Plasma.	Serum.	Serum.	Blood.	Serum.	Serum.
Number of examinations	90	9	16	81	85	83	14	8	14	18	18	18	4	10	19	8
„ animals	72	9	11	71	58	67	12	4	14	8	8	8	4	10	19	8
Average viscosity	*3·80*	*2·89*	*1·92*	*1·83*	*3·68*	*1·78*	*3·8*	*2·04*	*1·9*	*3·74*	*2·01*	*1·8*	*2·04*	*4·27*	*1·65*	*1·75*
Maximum	5·27	3·45	2·25	2·13	7·39	2·77	4·7	2·27	2·18	4·83	2·5	2·3	2·15	4·8	1·87	1·98
Minimum	2·95	2·55	1·73	1·55	2·7	1·49	3·05	1·85	1·72	2·7	1·7	1·6	1·87	3·8	1·5	1·58
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Total variation	61·1	31·2	27·4	31·8	127·3	72·1	43·5	22·7	24·3	57	39·8	38·9	13·7	23·4	22·4	22·8
Variation above average	38·7	19·4	17·2	16·7	100·6	56·06	32·7	12·4	14·7	29·2	24·4	27·8	5·4	12·4	13·3	13·1
„ below „	22·4	11·8	10·2	15·1	26·7	16·06	10·8	10·3	9·5	27·8	15·4	11·1	8·3	11	9·1	9·7
Percentage of values above and on average	41·1	44·5	37·5	37	40	51·8	57·2	87·5	43	55·5	50	55·5	50	40	52·5	37·5
Percentage of values below average	58·9	55·5	62·5	63	60	48·2	42·8	12·5	57	44·5	50	44·5	50	60	47·5	62·5
Percentage of values above normal average	—	—	—	—	34	35	57	37	79	—	—	—	—	—	—	—
Percentage of values below normal average	—	—	—	—	66	65	43	63	21	—	—	—	—	—	—	—

The following table shows the mathematical relations between the viscosities of blood, plasma, and serum :—

	Normal Horses.	Horses Suffering from Horse-sickness.	Immune Horses.	Normal Donkeys.	Normal Sheep.
$\frac{\text{Viscosity of blood}}{\text{Viscosity of serum}}$ =	2·08	2·07	2·0	2·08	2·59
$\frac{\text{Viscosity of blood}}{\text{Viscosity of plasma}}$ =	1·98	—	1·86	1·86	—
$\frac{\text{Viscosity of plasma}}{\text{Viscosity of serum}}$ =	1·05	—	1·07	1·12	—

If we take the viscosity of serum = 1 we obtain the following table :—

	Serum.	Plasma.	Blood.
Normal horses	1	1·05	2·08
Horses suffering from horse-sickness	1	—	2·07
Immune horses (against horse-sickness) ..	1	1·07	2·0
Normal donkeys	1	1·12	2·08
Normal	1	—	2·59

The figures of the three tables above are expressions of the following biological facts :—

I. Blood.—The internal friction of the entire blood of each species examined is much higher, nearly double, that of the blood liquid (the plasma) and a little more than double that of serum. At first sight it appears that the cause of this phenomenon is the presence of blood corpuscles. A certain relation between volume of blood corpuscles and viscosity of blood is shown by the following comparison of the respective figures :—

	Viscosity of blood.	Volume % of blood corpuscles.
Normal donkeys ..	3·74	33
Normal horses	3·8	33·8
Normal sheep	4·27	40·3
Sick horses	3·68	34·3
Immune horses ..	3·8	31·1

Comparing horses, donkeys, and sheep, the viscosity of the blood which contains the greatest quantity of blood cells is highest. On the other hand, comparing the values of sick and immune horses, we note a slight disproportionality between internal friction and volume of blood corpuscles. The number of erythrocytes is, of course, though the main, not the only factor of the internal friction of blood under pathological conditions. Further specially

arranged quantitative experiments, demonstrating the influence of the concentration of red blood corpuscles on the internal friction of a system, are the following :—

1. *Suspension of Erythrocytes in Serum.*

Vol. of corpuscles. %	Number of corpuscles in millions.	Viscosity of the suspension.
40 ·0	8 ·4	4 ·5
36 ·6	7 ·5	3 ·95
31 ·1	6 ·55	3 ·5
26 ·7	5 ·6	3 ·15
22 ·2	4 ·65	2 ·8
17 ·8	3 ·75	2 ·45
13 ·3	2 ·8	2 ·15
Serum alone	—	1 ·8

Remark.—Temperature 23 C. Defibrinated blood from horse 3250, previously concentrated by centrifugalising and then diluted with serum from the same horse.

2. *Suspension of Erythrocytes in Physiological Water.*

Vol. of corpuscles. %	Number of corpuscles in millions.	Viscosity of the suspension.
56 ·0	11 ·9	5 ·8
50 ·7	10 ·8	4 ·55
47 ·5	10 ·1	4 ·6
39 ·0	8 ·3	3 ·5
32 ·5	6 ·9	2 ·83
29 ·0	6 ·2	2 ·7
25 ·8	5 ·5	2 ·35
21 ·2	4 ·5	2 ·2
17 ·0	3 ·6	1 ·8
13 ·5	2 ·9	1 ·5
10 ·0	2 ·1	1 ·33
Physiological water	—	1 ·0

Remark.—The blood corpuscles were washed twice with 0 ·9 per cent. Na Cl. The number of corpuscles was found by division of the volume by the constant figure 4 ·7 which was derived from a great number of parallel examinations of volume and number of erythrocytes.

Graphically shown—ordinates representing the viscosity of the suspension, abscissae its content of erythrocytes—both series of figures give curves which are slightly concave towards the abscissae.

The following table contains the average values of the volume of blood corpuscles in ascending order with the respective average viscosities from various examinations on normal horse blood :—

Volume of blood corpuscles. %	Viscosity.	Volume %. Viscosity.	Volume % blood corpuscles calculated.
23	2 ·81	8 ·2	—
24	2 ·7	8 ·9	—
25	3 ·18	7 ·9	25 ·1
27	3 ·31	8 ·2	27 ·4
28	3 ·33	8 ·4	27 ·8

Volume of blood corpuscles. %	Viscosity.	$\frac{\text{Volume \%}}{\text{Viscosity.}}$	Volume % blood corpuscles calculated.
29	3·32	8·7	27·6
30	3·43	8·75	29·4
31	3·45	9·0	29·8
32	3·62	8·85	32·7
33	3·66	9·0	33·5
34	3·66	9·3	33·5
35	3·80	9·2	36·1
36	3·78	9·5	35·7
37	3·82	9·7	36·5
38	4·13	9·2	—
39	4·11	9·5	—
41	4·56	9·0	—

On a curve-table the viscosities from 3·18 to 3·82 lay on a straight line, and accordingly the quotient $\frac{\text{Vol.\%}}{\text{Viscos.}}$ is almost regularly increasing, hence it is possible to calculate the volume of the blood corpuscles from the viscosity by means of the simple formula—

$$\text{Volume \%} = (\text{Viscosity})^2 \times 2{\cdot}5.$$

As it can be seen in the last column of the table, this formula permits of calculating the volume of blood cells with satisfactory approximation.

The very close relation, almost proportionality, between quantity of erythrocytes and internal friction of the blood enables us to obtain indications on the degree of eventual destructions of blood cells in the organism by measurement of the viscosity of the blood. On the other hand, when we find a subnormal viscosity a diminution of erythrocytes must have taken place. This deduction can be elucidated by means of three examples.

1. *Haemolysis.*—In all horses suffering from haemolysis due to the injection of an isolytic serum the percentage of the volume of blood corpuscles and the viscosity of the blood were lower than normal.

Horse 3028.

	November, 1907.			December, 1907.							
	28.	29.	30.	1.	2.	3.	4.	5.	6.	7.	8.
Viscosity of blood... ...	—	—	2·7	2·75	2·9	3·0	2·9	2·95	2·7	2·75	Died.
Volume % of blood corpuscles	—	—	—	17·0	19·0	17·0	14·0	15·0	15·0	21·0	—
Clinical Remarks ...	Urine red, conjunctiva yellow.	Conjunctiva yellow. Urine dark red, contains blood corpuscles.	Urine brown, corpuscles.	Conjunctiva yellow. Urine normal.			Conjunctiva normal.				

Horse 3126.

Viscosity of blood	—	—	3·25	2·95	2·9	3·2	2·95	2·9	3·1	3·0	Recovered.
Volume % of blood corpuscles	—	—	—	13·0	12·0	15·0	13·0	13·0	14·0	14·0	—
Clinical Remarks ...	Conjunctiva yellow. Urine red, contains blood corpuscles.			Urine red.		Conj. nearly normal. Urine normal	Conjunctiva normal. Urine dark red.			Urine normal.	

2. *Artificial Anaemia.*—From the following horses the quantities of blood opposite their numbers had been drawn during a month :—

Horse 2959 .. .. .. 14·000 c.c.
Horse 2110 .. .. .. 24·000 c.c.
Horse 2603 .. .. .. 24·000 c.c.

Examinations made six days after the last tapping :—

Horse.	Volume of Blood Corpuscles.	Viscosity of.		
		Blood.	Plasma.	Serum.
2110	20·5	2·4	1·6	1·5
2603	22·5	2·45	1·55	1·46
2959	22·5	2·7	1·75	1·54

Loss of blood amounting to about a quarter of the total blood quantity of an animal causes a considerable decrease of the blood viscosity. (Compare later the tables of experiments on serum-horses.)

3. *Equine Piroplasmosis.*

This disease is accompanied by a breaking down of red blood cells, and we find, of course, similar results as above. (See tables on piroplasmosis later.)

Stagnation (stasis) of the blood in the body increases the quantity of carbonic acid in the blood and also its viscosity and the volume and number of erythrocytes (several authors). We must be very careful, therefore, when experimenting that in taking the blood from the jugular vein no stasis is caused by pressure.

The following experiments were made :—

	Volume of Blood Corpuscles.	Viscosity of.		
		Blood.	Plasma.	Serum.
	%			
1. Horse 3256, normal	34	3·95	2·0	1·75
Fifteen minutes' gallop, five minutes vein strangulated	43	5·5	—	—
2. Horse Nera, normal	—	4·6	—	—
Vein five minutes strangulated	—	7·0	—	—
After fifteen minutes' gallop, no strangulation	—	5·4	—	—
Fifteen minutes' gallop, then strangulation for three minutes	—	7·2	—	—
3. Horse 3249, normal	46	4·95	—	—
Vein strangulated for five minutes ..	53	6·7	—	—
4. Horse 3260, normal	32	3·7	1·9	1·7
Fifteen minutes' gallop, then vein strangulation for five minutes	—	5·08	2·0	1·9
5. Horse 3260. (*a*) Defibrinated blood	30	3·15 of def. blood.	—	—
Twenty minutes' treatment with CO_2 ..	30·5	3·2 of def. blood.	—	—
(*b*) Defibrinated blood	28·8	3·4 of def. blood.	—	—
Twenty minutes' treatment with CO_2 ..	33·0	3·6 of def. blood.	—	—

By stopping the flow of blood (1) the volume of the corpuscles increases and, of course, (2) the viscosity of the entire blood, and (3) the viscosities of plasma and serum increase. These results correspond with those of Limbeck. Hamburger, and others, viz., that by the introduction of CO_2 into defibrinated blood the volume of the erythrocytes increases and that the serum becomes richer in albumen, sugar, and fat. We must consider that this latter fact is the cause of the rise of internal friction in our experiments with stagnated blood, plasma, and serum, and that the accumulation of carbonic acid in the living animal by the stagnation is also responsible for the increase of viscosity of blood, plasma, and serum. The highest value of blood viscosity can be observed in very severe cases of horse-sickness, when pulse and respiration are strongly affected. In these cases generally dikkop is present.

As a rule it is a bad prognosticum when viscosity and volume of corpuseles rather suddenly increase; it mostly points to death. On the other hand again death may occur without having been previously signalised by increase of the viscosity of blood.

The question as to how the red blood corpuscles can increase the internal friction of a system so considerably is of physical and pathologieal interest.

There are three possibilities:

1. The erythrocytes moving forward leave vortiees in the medium behind them, and these are obstacles for the straight movement of the following globules.

2. The cells make a double movement (like a wheel)—(*a*) straight forward in the direction of the main stream, (*b*) round their own axis in any possible direction. This latter motion again takes parts of the liquid, attaehed by adhesive forces with it, and thus produces small secondary streams which might be opposed to the main stream.

3. On the enormous total surfaee of the erythroeytes—as on every surfaee—condensations and absorptions take plaee. These proeesses are variable and dependent on the membrane and contents of the eell and also on the physico-ehemieal properties of the outside liquid, i.e. briefly on the kind and concentrations of electrolytes and physieal properties of the colloids in the plasma. (See chapter on surface tension.) Condensation causes an inerease of the internal friction, and, since the physieo-chemical properties of serum and plasma are considerably altered in horse-siekness and piroplasmosis (see tables later), we find under pathological condition alterations of the viscosity of blood, which are not simply eorresponding with the alteration of the volume of the red blood corpuscles, because the physieal eonditions for absorption and condensation are ehanged.

The following experiments prove that not only the absolute extent of surface, but also the properties of the membrane of the erythroeytes influenee the internal friction of the suspension:—

		Viscosity.
26/11/07	Defibrinated blood, horse 3161, filtrated	**3·7**
	Centrifugalised. Serum cleanly taken off	1·9
	Deposit filled up with 0·9 Na Cl to original volume, mixed. Suspension	2·65
	Centrifugalised. Liquid taken off	1·08
	Deposit filled up as above. Suspension	2·7
	Centrifugalised. Liquid taken off	1·0
	Deposit filled up as above. Suspension	2·65
	Centrifugalised. Liquid taken off	1·0
	Deposit filled up with the serum	**3·45**
13/11/07	Defibrinated blood, horse 2915, filtrated	**4·0**
	Centrifugalised. Serum eleanly taken off	1·8
	Deposit filled up with physiol H_2O to original volume, carefully mixed. Suspension	2·4
	Centrifugalised. First washing liquid taken off	1·05
	Deposit filled up as above. Suspension	2·4
	Centrifugalised. Seeond washing liquid taken off ..	1·0
	Deposit filled up as above. Suspension	2·22
	Centrifugalised. Third washing liquid taken off	1·0
	Deposit filled up with the serum	**3·35**
	Centrifugalised serum	1·7
	Physiological water	1·0

The defibrinated blood, which is made with washed corpuscles, has a much lower viscosity than the original defibrinated blood. I consider, therefore, that by the washing process the membrane is so altered that its adhesion to the serum decreases. The isotonic salt solution (0·9 per cent. Na Cl) does not affect the volume of the globules (Hamburger) and subsequently not the total extent of surface, but it creates new irreversible conditions of the membrane with regard to absorption and condensation.

II. Defibrinated Blood has a viscosity which is about a quarter lower than that of original blood. Blood is physically a suspension of erythrocytes in plasma, defibrinated blood a suspension of the cells in serum ; the difference between defibrinated and not defibrinated blood lies in the presence or absence of the fibrine-forming components. Notwithstanding this the differences between the viscosities of defibrinated and not defibrinated blood on one side and of serum and plasma on the other side are not equal. The latter difference of the internal friction (between plasma and serum) is about ten times smaller than that between whole blood and defibrinated blood. The reason is probably the destruction of blood corpuscles by the manipulation of defibrinating, and the loss of these cells which are retained by the fibrine by absorption or adhesion and by the paper or muslin filter.

III. Plasma.—The internal friction is very little higher than that of serum, the limits of variations are much narrower, the latitudes being only about half of those of the viscosity of blood (for horses).

IV. Serum is blood without corpuscles and fibrine components and its internal friction is, of course, comparatively low, and its variability is also small. The limits of variations to the viscosities of blood and serum of horses suffering from horse-sickness are about twice as wide as those of normal animals. Roughly speaking, serum consists of water, salts, and proteids. It is especially the latter which dominate the internal friction of the system. After elimination of the majority of them by boiling the viscosity of serum is considerably decreased, as the following experiments demonstrate :—

		Viscosity.
13/2/08	Serum horse 3249, normal	1·7
	Boiled, coagulum filtered off, liquid	1·18
	Serum 2904 (normal horse)	1·8
	Boiled, filtrated liquid	1·3
16/11/07	Serum horse 2917, normal	1·8
	Heated at 80° C., filtrated liquid	1·55

A smaller amount of albuminoids is precipitated at 80° C. than by boiling, therefore the remaining liquid (which looks slightly opalescent) has still a remarkable viscosity.

Ions have undoubtedly an influence—like on other peculiarities of colloids—so on their internal friction, but the role of each ion of the serum in this respect has not yet been thoroughly studied.

Heat, especially when acting for a certain time, greatly affects the serum-colloids, thus altering the viscosity of the system :

		Viscosity.
30/9/07	—1.—Serum horse 2915, normal	1·75
	Heated half an hour at 60° C.	5·25
	Serum horse 2917, normal	1·75
	Heated half an hour at 60° C.	4·10

	Viscosity.
8/10/07—2.—Serum horse 2915, normal	1·87
Heated half an hour at 45° C.	1·86
,, ,, 50° C.	1·9
,, ,, 55°–58° C. ..	2·4
Serum horse 2917, normal	1·72
Heated half an hour at 45° C.	1·75
,, ,, 50° C.	1·8
,, ,, 55°–58° C. ..	2·0
9/10/07—3.—Serum horse 2915, normal	1·7
Heated half an hour at 55°–56°	2·05
,, ,, 60°	4·2
Serum horse 2917, normal	1·7
Heated half an hour at 55°–56°	2·0
,, ,, 60°	2·8

The effect of the heat differs on various sera, though they have been treated exactly in the same way.

These results are in accordance with those of French authors, who also observed an increase of viscosity (and opalescence) when inactivating serum by heat.

(Effect of heat on surface tension and co-efficient of optical refraction, see later.)

The influence of the concentration of the OH ion in the serum is shown by the following preliminary experiments (order of figures: increasing OH concentration) :—

	Viscosity.	Surface tension. 37°
11/5/08—1.—Serum horse 3524. T=21°.		
5 c.c. + 5 drops n. H_2SO_4 acid reaction, opalescent	5·4	5·31
Serum normal	1·90	6·14
5 c.c. Serum + 5 drops nKOH	1·83	6·14
2/6/08—2.—Serum horse 3590. T=21°.		
4 c.c. Serum + 2 drops $\frac{n}{2}$ H_2SO_4 acid reacting	2·57	4·94
4 c.c. Serum + 1 drop $\frac{n}{2}$ H_2SO_4 very slight alkaline reaction	2·33	5·92
Serum normal	2·12	5·98
4 c.c. + 4 drops n Na OH	2·13	5·79
23/6/08—3.—Serum horse 3647. T=22°.		
5 c.c. Serum + 2 drops $\frac{n}{2}$ H_2SO_4 very slight alkaline reaction	1·95	5·07
Serum normal	1·83	5·82
5 c.c. Serum + 2 drops nKOH	1·85	5·93
5 c.c. Serum + 4 drops nKOH	1·88	5·85

In the last two experiments the viscosity is minimum at the natural concentration of the hydroxylion, and is greater at higher and lower alkalinity. The evident relation between internal friction and alkalinity seems to be reverse proportionality. The fact that the internal friction of the blood liquid is lowest at the natural alkalinity would be the most advantageous condition from the standpoint of haemodynamics.

Further experiments have to be made to draw definite conclusions.

Experiments with Colloids.

I mentioned that it is the colloidal nature of serum which causes its high viscosity compared with water. It has also been stated that the viscosity of blood is chiefly due to the globules, and I tried to explain the manner of their influence. The internal friction of blood and serum is considerably altered in diseases; but it is not sufficient for the physiologist or pathologist to make the statement that it is so, but the reason should also be known Once we know the factors which create and alter a biophysical state we are able to recognise them in a complex of physico-pathological symptoms and then to find means to eliminate them. For instance, we know that a subnormal viscosity of blood is a sign of anaemia, or that a supernormal value of internal friction points to heart weakness. This knowledge indicates directions for the treatment.

With regard to serum, the conditions are not so simple. Concerning the factors of the viscosity of serum only the following is known :—

(1) The internal friction is dependent on the concentration and actual state of the colloids, and

(2) Ions and non-electrolytes influence the viscosity of the colloids, and the viscosity changes according to variations of the former.

(3) The disappearance of serum components, or the rise of new substances in it, under pathological circumstances, will alter the internal friction. (Bile pigments, urine components, haemoglobin, microbes, and their metabolic products.) The individual influence of these factors has not yet been ascertained. As the serum is already an enormously complicated heterogeneous system I experimented on a simple colloid, such as gelatine, to get an idea of the principles of the influences of concentration of the colloid and of some ions on the internal friction (and surface tension) of a heterogeneous system.

(1) *Gelatine.*

1·5 % Gelatine (=100 %) diluted with dist. H_2O. T=27°.

12/12/07.

Concentration of gelatine	0 H_2O	10	20	30	40	50	60	70	80	90	100
Viscosity ..	1	1·1	1·25	1·5	1·55	1·65	—	2·0	2·4	2·55	2·55

The internal friction of this colloid is a linear function of the concentration from 0 to 1·5 per cent.

(2) *Globuline.*

Serum diluted with H_2O in the proportion of 1 : 10. The globulines precipitated are washed several times and then dissolved in $\frac{n}{2}$ NaOH. T=27°.

13/12/07.

Concentration of globuline	0 (NaOH)	0·5	1·0	1·5	2·0	2·5	3·0
Viscosity	1·13	1·23	1·3	1·37	1·43	1·51	1·6

The internal friction of alkaline serum globuline is also a linear function of the concentration within 0 to 3 per cent.

(3) *Gelatine + Saponine.*

2 per cent. Gelatine—Viscosity 4·0. 4 per cent. Saponine—Viscosity 1·2. T = 27°.
12/12/07.

Parts of Gelatine.	Parts of Saponine.	Viscosity of mixture.
0	10	1·2
1	9	1·5
2	8	1·7
3	7	1·9
4	6	2·1
5	5	2·5
6	4	2·65
7	3	3·0
8	2	3·25
9	1	3·4
10	0	4·0

The viscosity of the mixture increase continually with the increasing concentration of gelatine; or in other words, saponine decreases the internal friction proportionally to its quantity, but quicker than water does, for the curves are convergent towards the concentration 0 of gelatine.

(4) *Serum + Colloidal Fe $(OH)_3$.*

11/10/07.

Serum c.c. ..	1	1	1	1	1	1	1	1	1
Fe $(OH)_3$ drops ..	2	4	6	8	10	12	14	16	18
Viscosity ..	1·7	1·6	1·55	1·5	1·5	1·5	1·45	1·45	1·4

Serum alone .. .. 1·8
$Fe_3(OH)$ alone .. .. .. 1·0

This curve differs from that of viscosity of the gelatine–saponine mixture. The first drops of $Fe_3(OH)$ decrease the viscosity more considerably than the following. Ferrihydroxyd is, of course, a colloid of a quite different kind than saponine, gelatine, or globuline.

Influence of Anions.

24/10/07—1.—Half per cent. neutral gelatine. T=25°.

$+ \frac{n}{2}$ salt solution.

	Cl.	Br.	I.	NO_3	SO_4.
Viscosity, Na	1·5	—	—	1·52	1·8
K	1·45	1·52	1·4	1·48	—

Half per cent. gelatine = 1·82.

Na-Anions decrease the internal friction of neutral gelatine in the descending order—

Cl. NO_3. SO_4.

K-Anions decrease in the descending order.

I Cl. NO_3. Br.

20/5/08—2.—(*a*) 1 per cent. slightly alkaline gelatine T=23°.

$+ \frac{n}{6}$ salt solution.

(*b*) 1 per cent. slightly acid gelatine.

$+ \frac{n}{6}$ salt solution.

Na-Anions.	NO_3.	Cl.	SO_4.	Acetate.
Viscosity (*a*) ..	1·76	1·81	1·85	1·93
(*b*) ..	1·53	1·62	1·7	1·79

Controls: Alkaline gelatine .. .. 2·10
Acid ,, ... 2·13
Neutral ,, ... 1·75

These anions decrease the internal friction of acid and alkaline gelatine in the descending order—

NO_3. Cl. SO_4. Acetate.

The order is not reversed with the change of reaction, as is the case with the order of influence of ions on surface tension (*vide* later).

The decreasing effect of the anions on viscosity is much greater in the acid than in the alkaline gelatine.

The viscosity of acid and alkaline gelatine is much higher than of the neutral colloid.

C.—SURFACE TENSION OF SERUM.*

I.

Surface tension or surface pressure is a force acting on the surface of every liquid and solid. It has the tendency of making the surface as small as possible, and therefore produces the globular form of a drop of liquid.

The surface pressure is the cause of capillary attraction and capillary depression, because it is higher on a convex, and less on a concave meniscus compared with the plane surface.

A certain relation exists between surface energies and chemical energies, hence if the surface tension of a liquid chemical compound is known, its molecular weight can be calculated. Forces of curved surfaces also influence the osmotical equilibrium, for instance, if we put a tube in a salt solution, the wall of which is easily permeable for the solvent and the dissolved body, the concentration of the latter one is always higher on the concave side of the surface, viz., in the tube (Kauflert). Applying this theory to the organisms, the salt concentration in the cells is always higher than in the interstitial liquid, or the osmotical equilibrium for instance, between red blood cells and plasma, is not the same as it would be *ceteris paribus* in vitro with a plane separating surface.

On every surface of either liquids or solids, condensations of the neighbouring substances take place. This is called *adsorption* or *absorption*. Every absorption is dependent on the decrease of the surface tension of the absorbens by the absorbed substance (Gibbs‡).

Coal dust, powdered platinum or palladium absorb enormous quantities of gas; they also absorb dissolved bodies out of a solution. The absorbed quantity is naturally absolutely greater the larger the total absorbent surface. The importance of these facts for physiology is evident if we remember the enormous total surface the cells of the organism have, and that it is, for instance, for the total number of erythrocytes of a human being about 2800 square metres.

The eminent role of surface energies for heterogeneous systems, such as colloids, is elucidated by the fact that 1 c.c. of a substance which as a cubus has a surface of 6 square cm., if split up in particles of 0·1μ diameter would have a total surface of 60·000 square metres.§

* Appeared in the *Transvaal Medical Journal*, August, 1908.
† Kaiserl. Akad. d. Wissenschaften Wien, math. nat. klasse. *43*, 686, 1902.
‡ Transact., Connecticut Academy. *3*, 108, 343.
§ *Wolfgang Ostwald*, Zeitschrift fur Chemie und Industrie der Kolloide, *1*, 291, 1907.

Since we know that the *immune substances* and the *enzymes* are *colloidal* we understand that the visible and measurable part of their respective reactions is more like an absorption than a chemical reaction.

If we now take a physiological colloidal system in view, we have to distinguish

(*a*) surface tension on the surface of the whole system (in vitro) towards air; phases: *liquid-gas* (the liquid itself is a pluriphasic system);

(*b*) surface tension of each colloid particle towards the medium; phases: *"solid"-liquid* (the liquid is a salt solution, monophasic system).

What are the consequences of this physical arrangement?

Ad. a. Colloid particles are condensed in the surface, so to say, absorbed by it, whereby the surface tension decreases to a certain minimum. The result is that the density of the system with regard to colloid particles is higher in the surface than in the interior of the liquid. It has to be borne in mind that this is the case not only in the systems liquid-air, but also in liquid-solid, that is to say, anywhere in the organism where the body fluids are in contact with cell walls. The degree of condensation is dependent—amongst other factors—on the substances on both sides of the surface, and upon the radius of its curve.

Condensation of colloid particles which follows a decrease of surface tension takes place in

(1) *Haemo- and bacteriolysis*; absorption of lysines and anti-bodies on the curved surface of the blood and microbe-cell respectively.

(2) *Phagocytosis*; the leucocytes condense microbes or other particles on their surface. This absorption is perhaps the last phase of a chemotactic process.

(3) *Action of enzymes*; the enzymes are colloid particles and absorb crystalloids and colloids on their surface. The condensation, i.e. increase of concentration is the main cause of the acceleration of the reaction.

The ability of some colloids—for instance, proteids—of superficial condensation to such a degree that *membranes* are formed causes a still more pronounced separation of colloid-complexes, viz., isolation and individualisation of each complex, each cell.

The condensed surface already influences the passage of crystalloid molecules and ions. But the membrane completely acts as a typical separator and regulator for the transition of substances from one to the other side of the surface wherein it is localised. The surface tensions, or more precisely, the difference of surface tensions of two systems in contact with each other, creates the conditions for the formation of membranes (*Devaux*,* *Ramsden*,† *Metkalf*‡). *The membranes dominate and regulate the entrance into and exit from the cells and thus influence the protoplasmic metabolism by their semi-permeability or elective permeability* (*Zangger*§).

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* Procés verbaux des sciences Bordeaux, 1903-4.
† Proc. Royal Soc. 72, 156, 1903.
‡ Zeitschr. Physik. Chemie. 52, 1, 1905.
§ Ergebnisse d. Physiologie v. Asher-Spiro. 7, 99, 1908.

Ad. b. Also on the surface of each particle condensations must take place. Crystalloid molecules and ions of the medium especially are absorbed and thus influence the properties of the surfaces of the particles.

On the other hand the properties of the entire system will be dependent on the peculiarities of the single globules, namely:

(1) *The internal friction* of a colloid solution is (besides the internal friction of the medium) undoubtedly influenced by the surface, the volume, and the degree of imbibition of the particles.*

(2) *The surface tension* of the system itself, as an expression of the concentration of the particles and their tendency to condense in the surface, will be affected as soon as this tendency is altered as a consequence of changes of the properties of the particles. As a matter of fact it is possible to give a colloidal solution another surface tension by the addition of electrolytes or non-electrolytes, which certainly are partially absorbed by the globules.

II. *Experiments.*

The following table shows the results of 108 examinations of surface tension of various horse sera in ascending order.

I examined (1) normal horses, (2) horses suffering from horse-sickness, (3) horses immune and hyperimmunised against horse-sickness (immune = passed through an attack of horse-sickness; hyperimmunisation = infusion of about 10 litres of blood from a highly sick into an immune horse), (4) serum horses, that is to say, hyperimmunised horses from which regularly large quantities of blood had been drawn in certain intervals (generally 6 litres at a time).

We see that the majority of values of surface tension range between 5·9 and 6·1, a large number between 5·7 and 5·9, and 6·1 and 6·2 respectively, and only a few below 5·7 and above 6·2.

Surface Tension of Horse Sera.

Date of Examination.	Number of Horse.	Remarks.	Surface Tension, 37°.
1908.			
May 12 ..	2901	Hyperimmunised: last bleeding eight days ago ..	4·87
June 20 ..	3411	Horse-sickness: climax	4·98
June 20 ..	3372	Horse-sickness; climax	5·10
June 22 ..	3084	Serum horse; last bleeding four days ago	5·23
June 22 ..	3663	Normal	5·37
July 2 ..	3146	Hyperimmunised six days ago	5·44
June 13 ..	1972	„ twenty days ago	5·44
„ 22 ..	3064	Serum horse: last bleeding four days ago	5·46
„ 24 ..	3685	Normal	5·49
„ 23 ..	3646	„	5·49
April 27 ..	3506	Normal	5·54
May 18 ..	3541	„ strangulated for three minutes	5·56

* Compare *Pauli and Handovsky*, Hofmeister's Beitrage. 11, 415, 1908.

Surface Tension of Horse Sera.—(Continued.)

Date of Examination.	Number of Horse.	Remarks.	Surface Tension, 37°.
1908.			
June 27 ..	3146	Hyperimmunised the day before; immediately before the second hyperimmunisation	5·57
May 18 ..	3541	Normal; strangulated ten minutes	5·66
June 20 ..	3608	Horse-sickness; climax seventeenth day	5·67
„ 22 ..	3033	Serum horse; last bleeding four days ago	5·67
July 2 ..	3076	„ „ five weeks ago	5·68
June 1 ..	3505	Horse-sickness; climax tenth day	5·69
„ 24 ..	3517	„ „ ninth day	5·69
May 3 ..	3049	Serum horse; immediately before bleeding	5·69
June 15 ..	3632	Normal	5·70
„ 3 ..	3591	„	5·72
April 30 ..	3364	„	5·73
June 20 ..	2903	Serum horse; last bleeding two days ago	5·75
„ 27 ..	3146	Hyperimmunised immediately after second hyperimmunisation	5·75
April 27 ..	3302	Horse-sickness begins; reaction ninth day	5·76
„ 27 ..	3465	„ „ „ „	5·76
June 23 ..	3637	Normal	5·76
„ 20 ..	3091	Serum horse; last bleeding two days ago	5·77
„ 27 ..	3119	Hyperimmunised day before; immediately before second hyperimmunisation	5·78
„ 15 ..	3625	Normal	5·78
„ 4 ..	3427	Horse-sickness; climax thirteenth day	5·79
„ 20 ..	2270	Serum horse; bled two days ago	5·79
June 23 ..	3638	Normal	5·80
May 4 ..	3512	„	5·81
June 27 ..	3119	Immediately after second hyperimmunisation	5·81
„ 26 ..	3079	Immune against horse-sickness	5·82
„ 23 ..	3647	Normal	5·82
April 27 ..	3447	Immune	5·83
June 13 ..	3451	Hyperimmunised	5·83
„ 29 ..	1288	„	5·83
July 2 ..	3119	„ six days ago	5·85
June 22 ..	3670	Normal	5·85
„ 24 ..	3675	„	5·87
April 27 ..	3499	Horse-sickness; climax ninth day	5·87
June 1 ..	3546	„ „ tenth day	5·87
„ 22 ..	3668	Normal	5·89
May 11 ..	3524	Normal	5·90
June 12 ..	3302	Horse-sickness; climax fourteenth day	5·91
„ 13 ..	1293	Hyperimmunised	5·91
July 1 ..	1288	„ one day after first bleeding	5·92
June 2 ..	3589	Normal	5·92
„ 1 ..	3551	Horse-sickness; climax	5·92
„ 17 ..	1288	Hyperimmunised; bled six days ago	5·93
April 28 ..	3507	Normal	5·93
June 17 ..	3449	Immune	5·94
„ 27 ..	1162	Hyperimmunised	5·94
„ 27 ..	3685	Normal	5·95
May 11 ..	3474	Horse-sickness; climax nineteenth day	5·95
May 18 ..	3538	Normal; strangulated for ten minutes	5·96
April 30 ..	3451	Horse-sickness; climax tenth day	5·96
May 11 ..	3457	„ „ fifth day	5·97
„ 11 ..	3445	„ „ ninth day	5·97
„ 18 ..	3538	Normal; strangulated for six minutes	5·98

Surface Tension of Horse Sera.—(Continued.)

Date of Examination.	Number of Horse.	Remarks.	Surface Tension, 37°.
1908.			
June 15 ..	3623	Normal	5·98
,, 2 ..	3590	,, ...	5·98
May 18 ..	3538	,, strangulated for three minutes	5·99
,, 3 ..	3510	,, ...	5·99
June 17 ..	1085	Hyperimmunised; first bleeding six days ago ..	5·99
June 3 ..	3473	Horse-sickness; climax fourteenth day	6·00
,, 24 ..	3682	Normal	6·00
,, 3 ..	3506	,, ...	6·01
,, 29 ..	1672	Serum horse; bled two months ago	6·02
,, 3 ..	3490	Horse-sickness	6·02
July 1 ..	1660	Serum horse; last bleeding day previous	6·03
June 20 ..	3172	,, ,, ,, two days previous ..	6·03
July 1 ..	1672	,, ,, ,, day previous	6·04
May 18 ..	3541	Normal	6·04
June 15 ..	3636	,, ...	6·06
April 28 ..	3504	,, ...	6·06
,, 28 ..	3342	,, ...	6·06
June 3 ..	3607	,, ...	6·07
,, 3 ..	3555	Horse-sickness	6·07
,, 17 ..	3408	Immune horse-sickness	6·07
,, 29 ..	1660	Serum horse; last bleeding four weeks ago	6·08
,, 1 ..	3264	Horse-sickness; climax	6·09
,, 1 ..	3588	Normal	6·09
,, 1 ..	3587	,, ...	6·09
May 3 ..	3511	,, ...	6·10
April 4 ..	3340	,, ...	6·10
June 3 ..	3590	,, ...	6·11
,, 15 ..	3630	,, ...	6·11
,, 13 ..	3584	Horse-sickness, end; eighth day	6·11
May 18 ..	3538	Normal	6·12
June 30 ..	1972	Serum horse; last bleeding twelve days ago	6·13
,, 30 ..	1293	,, ...	6·14
,, 1 ..	3424	Horse-sickness; climax	6·14
May 13 ..	3524	Normal	6·14
June 15 ..	3635	,, ...	6·16
April 30 ..	3317	,, ...	6·17
,, 30 ..	3362	Horse-sickness; climax	6·17
May 4 ..	2901	Hyperimmunised; bled thirteen days ago	6·19
June 9 ..	1288	Hyperimmunised	6·21
April 28 ..	3314	Normal	6·24
June 17 ..	3411	Immune	6·27
,, 1 ..	3591	Normal	6·27
,, 17 ..	1660	Hyperimmunised; first bleeding six days previous ..	6·28
June 3 ..	3589	Normal	6·45

The surface tension at 37° * of *normal* horse-serum varies from 5·37 to 6·45. The average from 42 examinations on 36 horses being *5·95*, the variations are 9·75 per cent. below and 8·4 per cent. above the average =18·15 per cent. total variation. The average surface tension of the sera of 23 different horses suffering from *horse-sickness* is *5·85*, that is to say, 1·68 per cent. lower than the average normal surface tension. But the

* The surface tension of water at this temperature is *7·132*. Hence the surface tension of serum is lower than that of water, but the viscosity is higher.

variations are in somewhat wider limits, namely, between 4·98 to 6·17 — 20·34 per cent. Horses with a very strong attack of horse-sickness, when circulation and respiration are highly altered, generally show a subnormal value of surface tension of serum. I am inclined to attribute this phenomenon merely to the stasis of the blood in the jugular vein and accumulation of CO_2, because it was possible to reproduce a decrease of surface tension of serum by artificial strangulation of the vein, as the following examples demonstrate :—

		Surface tension of Serum at 37°.	Viscosity of Serum at 23°.
18/5/08—1.—	Horse 3541, normal	6·04	1·96
	Three minutes' strangulation	5·56	2·08
	Ten minutes' strangulation	5·66	2·16
18/5/08—2.—	Horse 3538, normal	6·12	—
	Three minutes' strangulation	5·99	—
	Six minutes' strangulation	5·98	—
	Ten minutes' strangulation	5·96	—
		Surface tension of Blood at 37°.	Viscosity of Blood at 25°.
16/1/08—3.—	Horse 3256, normal	5·62	3·95
	A fifteen minutes' gallop and five minutes' strangulation	5·42	5·55

At the same time the viscosity of serum and blood increases*, and a combination of both methods therefore gives very valuable prognostical indications, especially when the examinations are made several times on different dates.

It is a well-known fact that by accumulation of carbonic acids, the microbicide properties of serum increase, a fact which is the base of the hyperaemia treatment by artificial stasis of the blood. At present it is not possible to define the role of the decreased surface tension in the destruction of microbes, but there is no doubt that the absolute value of surface tension of the blood liquid on one side and of the microbe on the other, or rather the difference of both, has an influence on the structure of the membrane of the bacillus and on the condensation (absorption) of the blood anti-bodies on its surface.

We are not permitted to say that the decrease of surface tension alone is the cause of the increase of destructive properties of a serum, as the following experiment shows :—

Serum Mule 3417.	Surface Tension.	Viscosity.
Normal	6·04	1·88
Heated for half an hour at 56°	5·85	1·9

The heating process is followed by a synchronical decrease of the surface tension and loss of the microbicide properties of the serum, for the alexines are destroyed. In this case the decrease of surface tension is rather a symptom of physical-chemical alterations of the serum colloids.

The influences of infusion (hyperimmunisation) and bleedings on the surface tension are shown by the following table :—

		Surface tension immediately before infusion of 5 litres of blood.	after
27/6/08.	Horse 3146	5·57	5·75
	„ 3119	5·78	5·81

* Compare W. *Frei*, on Viscosity of Blood. *Transvaal Medical Journal*, April, 1908.

		Surface tension immediately before bleeding.	Surface tension two hours after bleeding (6 litres).
18/6/08.	Horse 1293	5·79	5·89
	,, 1972	5·79	5·75
		1 day before.	1 day after.
29/6/08.	Horse 1288	5·83	5·92
1/7/08.	,, 1660	6·08	6·03
	,, 1672	6·02	6·04

The surface tension of serum is increased after *infusion*, whilst the viscosity is lower. That points to a decrease of serum-proteids. This is not easy to understand, because an increase in concentration had to be expected. But we must not forget that the figures only refer to serum of blood from the jugular vein, and that we have no indications about the blood which is probably accumulated in the distended capillaries of the intestines.* (Results of other transfusion experiments.)

In three instances *loss of blood* has a diminishing influence on the surface tension of the serum. The viscosity is also decreased. Both facts are probably due to the diffusion of water or intercellular liquid without, or with but very little, colloid substances into the blood vessels in order to keep the blood pressure at a normal height. The experiments are not yet numerous enough to draw definite conclusions.

After having observed that neutralisation of serum causes a considerable decrease of surface tension, the following experiments were made to study the influence of the concentration of the hydroxylion on the surface tension and viscosity :—

	Surface tension.	Viscosity.
11/5/08—1.—Serum horse 3524, normal	6·14	1·90
5 c.c. Serum + 5 drops nKOH ..	6·14	1·83
5 c.c. Serum + 5 drops n H_2SO_4 (acid reaction. Opalescent gelatinons) ..	5·31	5·4
Increase of OH concentration does not alter the surface tension, but causes a slight decrease of the viscosity. Change of reaction decreases the surface tension and considerably increases the viscosity.		
2/6/08—2.—Serum horse 3590, normal	5·98	2·12
4 c.c. Serum + 4 drops n Na OH ..	5·79	2·13
4 c.c. Serum + 1 drops $\frac{n}{2}$ H_2SO_4 (alkaline reaction)	5·92	2·33
4 c.c. Serum + 2 drops $\frac{n}{2}$ H_2SO_4 (acid reaction)	4·94	2·57
Increase and decrease of the OH concentration causes a diminution of surface tension (whilst the viscosity always becomes higher). The actual degree of alkalinity is the optimal one.		

* Compare *Tigerstedt*, Ergebnisse d. Physiologie. *6*, 303, 1907.

	Surface tension.	Viscosity.
2/6/08—3.—Serum horse 3589, normal	5·92	—
5 c.c. Serum + 2 drops n Na OH ..	6·01	—
5 c.c. Serum + 5 drops n Na OH ..	6·09	—
7 c.c. Serum + 2 drops $\frac{n}{2}$ H_2SO_4 (very slight alkaline reaction)	6·00	—
Increase and decrease of the number of OH ions causes an increase in surface tension. The actual concentration corresponds to a minimum of surface tension.		
23/6/08—4.—Serum horse 3647, normal	5·82	1·83
5 c.c. Serum + 2 drops nKOH ..	5·93	1·85
5 c.c. Serum + 4 drops nKOH ..	5·85	1·88
5 c.c. Serum + 2 drops $\frac{n}{2}$ H_2SO_4 (very slight alkaline reaction)	5·07	1·95

The optimal OH concentration is somewhat higher than the actual one

Conclusions.—The natural concentration of OH ions in normal horse serum is not always the optimal one for the maximum surface tension.

The alkalinity gives the surface tension a certain height which would not exist in a neutral or acid serum.

It follows from the experiments of *Buglia* * (who studied the influence of H concentration on cattle serum) that the surface tension shows two maxima, one of them coinciding with the natural OH concentration of serum, the other being immediately before the neutral zone at a lower degree of alkalinity.

Although the correlations between surface tension and *phagocytosis* have not been experimentally studied, we can safely say that a relation exists between (*a*) alkalinity, (*b*) surface tension of serum, and (*c*) intensity of phagocytosis, for it has been observed that the phagocytosis is a maximum at an optimal concentration of OH ions (not proportional) which is present in the serum.†

We know that there is an optimal OH concentration (or two optima) for the surface tension of serum, and it is interesting to find similar phenomena in a simple colloid, namely gelatine combined with various quantities of alkali.

30/5/08. Temperature =37° 0·75 per cent. gelatine.

Concentration of Na OH. %	Surface tension.
0	6·29
0·013	6·48
0·063	*6·63*
0·125	6·36
0·25	*6·58*
0·5	6·48
0·75	6·45
1·0	6·33

* Biochem. Zeitschrift. *11*, 311, 1908.

† *Hamburger* and *Hekma*. Bioch. Zeitschrift *9*, 273, 1908.

There are two optima, at the concentrations 0·063 and 0·25 respectively. The curve resembles *Buglia's*.

Another function of the hydroxylion, where an optimal concentration is also required, is the stabilisation of negative colloids (metal sols, *Bredig*).

It has yet to be found whether the points of greatest stability and greatest surface tension of a colloidal solution are coincident.*

Guided by the idea that the serum is a system of colloids with various electrolytes and non-electrolytes, and especially in order to obtain an idea of the effect of electrolytes, I commenced to study the influence of single ions on gelatine, and obtained the following results :—

I. 7/5/08.—One per cent. gelatine + Cl-kations in $\frac{n}{9}$ concentration.

	Surface tension 37°.	Viscosity at 25°.
Gelatine (control)	5·97	2·33
Gelatine + Na Cl	6·20	3·9
Gelatine + K Cl	6·29	2·15
Gelatine + Mg Cl_2	6·19	2·7
Gelatine + Ca Cl_2	6·34	3·35

The kations Na K, Mg Ca, with Cl as anion in $\frac{n}{9}$ concentration *increase* the surface tension of 1 per cent. neutral reacting gelatine in the descending order Ca K, Na Mg. *Whatmough* composed the order Ca Na, K Mg as increasing the capillarity of water. The viscosity increased in the order Na Ca, Mg K. (K decreases.)

II. 18/4/08.—One per cent. gelatine + Na-anions in $\frac{n}{5}$ concentration.

	Surface tension at 37°.
Gelatine (control)	6·24
,, +Na Cl	6·58
,, +Na_2 CO_3..	6·63
,, +Na_2 SO_4..	6·67
,, +Na NO_3..	6·47

Natrium anions in $\frac{n}{5}$ concentration *increase* the surface tension of 1 per cent. neutral gelatine in the descending order SO_4, CO_3, Cl, NO_3.

The same order of ions was stated to *increase* the surface tension of pure water, for Na in the same descending order SO_4, Cl, NO_3 (*Whatmough*†), and also the internal friction of H_2O in the descending order SO_4, Cl, NO_3 for several kations (*Wagner*‡).

III. 20/5/09.—5 c.c. 1 per cent. gelatine + 1 drop $\frac{n}{2}$ H_2 SO_4 + Na-anions in $\frac{n}{6}$ concentration.

* OH ion stabilises (disseminates) negative colloids and increases their surface tension, that means it prevents the particles from condensing in the surface. Also other anions espeeially citrate, oxalate, acetate, picrate have a stabilising effect. Citrate disseminates Ba SO_4 suspension (*Gengou*) and protracts the haemolysis by cobra venom (*Gengou*) ; that would be, it prevents the particles of the colloidal venom from condensing on the surface of the blood corpuscle.

† Zeitschrift Physik. Chemie. *39*, 129, 1902.

‡ Zeitschrift Physik. Chemie. *5*, 31, 1890.

	Surface tension 37°.	Viscosity at 23°.
Gelatine (neutral control ..	6·30	1·75
„ (sour control) ..	6·14	2·13
„ + Na Cl	6·05	1·62
„ + $Na_2 SO_4$	6·16	1·7
„ + Na NO_3	6·31	1·53
„ + Na $C_2H_3O_2$ ..	6·38	1·79

VI. 20/5/08.—One per cent. gelatine + 1 drop nKOH + Na-anions in $\frac{n}{6}$ concentration.

	Surface tension 37°.	Viscosity at 23°.
Gelatine (control neutral) ..	6·30	1·75
„ (control alkaline) ..	6·53	2·1
„ + Na_2CO_3	6·47	1·83
„ + Na NO_3	6·48	1·76
„ + Na_2SO_4	6·51	1·85
„ + Na Cl	6·53	1·81
„ + Na $C_2H_3O_2$ ..	6·57	1·93

The Na-anions Cl, (CO_3), NO_3, SO_4, acetate in $\frac{n}{6}$ concentration *decrease* the surface tension of 1 per cent. alkaline gelatine (except acetate) in the descending order (CO_3), NO_3, SO_4, Cl, whilst these ions *increase* the surface tension of acid gelatine in the same descending order Acet., NO_3, SO_4, Cl. (Cl decreases.) The order of influence of these ions is reversed as soon as the reaction is changed.

The differences of the effects of these anions are much greater in an acid than in an alkaline medium. In analogy to serum, the surface tension of alkaline gelatine is higher, of acid gelatine lower than of the neutrally reacting colloid.

The internal friction of acid and alkaline gelatine is *decreased* in the ascending order Acet., SO_4, Cl, NO_3, that is to say by the same ions which *increase* the viscosity of water in the ascending order NO_3, Cl, SO_4.

Résumé.

The surface tension is a factor of enormous biological importance, especially for the phenomena of haemo- and bacteriolysis, phagocytosis and enzyme reactions.

The surface tension of serum of horses suffering from horse-sickness is subnormal.

Infusion of homogeneous blood and considerable loss of blood have an influence on the surface tension of horse serum.

The alkalinity of the serum guarantees a certain height and probably stability of the value of surface tension, but this latter one increases not proportionally with an increase of the hydroxylion-concentration; there is a certain optimum (perhaps two optima), and when this is surpassed the tension decreases again.

The anions SO_4, Cl, NO_3 increase the surface tension of neutral gelatine in exactly the same the kations Na, K, Mg Ca nearly in the same order in which the capillarity of water is increased by them.

The order of influence of the Na-anions NO_3, Cl, SO_4 on gelatine is reversed as soon as the reaction is changed. The degree of influence of these ions is much higher in the acid than in the alkaline gelatine.

The surface tension of neutral gelatine (and neutral serum) is increased by the OH and decreased by the H-ion.

D.—ELECTRIC CONDUCTIVITY.

In a homogeneous system the electric conductivity is an expression of the number of free ions and thus of the degree of dissociation, and of the velocity of the ions.

In heterogeneous systems with electrolytes the resistance for the passage of the electric current is greater than it would be *ceteris paribus* in the electrolyt solution without suspended globules, for the latter

(*a*) absorb on their surface a certain number of ions and thus simply reduce the number of carriers of electricity;

(*b*) are mechanical obstacles for the migration of the remaining free ions.

Therefore the result of examination of the serum conductivity is dependent on

(*a*) number and kind of serum ions;

(*b*) quantity and actual state of serum colloids.

That is to say on two factors which are in competition with each other.

It was found that the conductivity of milk is considerably increased by elimination of the colloidal casein * (coagulation by means of the specific enzyme).

Similarly the colloidal ferrihydroxyde decreases the conductivity of NH_4Cl.†

The following experiments were carried out to study quantitatively the influence of various organic colloids in different concentrations on the conductivity of the same electrolyte, that is to say, the influence of albumine, globuline, gelatine, and saponine on the ions Na and OH. Albumine and globuline are colloidal components of serum wherein also both ions are present.

(1) *Albumine.*

Serum diluted with dist. H_2O in the proportion of 1 : 10. The globulines precipitate; the clear remaining liquid is boiled after addition of a small quantity of $(NH_4)_2\,SO_4$. The coagulum obtained is washed several times by means of dist. H_2O and centrifugal power until the liquid has a conductivity of $0{\cdot}75 \times 10^{-4}$. From the dried deposits a 4 per cent. stock solution is made with $\frac{n}{2}$ Na OH.

9/12/07. T=37°.

Concentration of Albumine. %	Conductivity $\times 10^{-4}$.	Differences, absolute.	Difference in percentages. %
$0 \cdot \left(\frac{n}{2}\text{ Na OH}\right)$	1097		
0·5	1056	41	3·7
1·0	1015	41	3·9
1·5	977	38	3·7
2·0	942	35	3·7
2·5	905	37	3·9
3·0	870	35	3·9
3·5	837	33	3·8
4·0	802	35	4·2

* *Schnorf*, Thesis, Zürich, 1904.

† *Dumanski*, Zeitsche, f. Chemie und Indust., I. Kolloide, *1*. 281, 1907.

The decrease of conductivity caused by increase of the colloid concentration amounting to 1 per cent. is about 7·7 per cent.

The remaining 4 per cent. stock solution is sterilised by boiling and kept overnight in the ice-chest.

10/12/07.

Conductivity of stock solution $786 \cdot 10^{-4}$; that is to say, a decrease of conductivity amounting to 16, equal to about 2 per cent., had taken place which I am inclined to attribute to absorption of ions by the colloid particles.

The same series is made as the previous day. The conductivity of the stock solution remains constant during the experiment:

Time.	Conductivity $\times 10^{-4}$.
10.30 a.m.	785
10.45 a.m.	787
4 p.m.	785
4.15 p.m.	786

10/12/07. T = 37°.

Concentration of Albumine. %	Conductivity $\times 10^{-4}$.	Differenees, absolute.	Difference in percentages. %
$0 \cdot \left(\frac{n}{2}\text{Na OH}\right)$	1095		
0·5	1055	40	3·7
1·0	1015	40	3·8
1·5	974	41	4·0
2·0	935	39	4·0
2·5	898	37	4·0
3·0	860	38	4·2
3·5	823	37	4·3
4·0	786	37	4·5

A difference in the colloid concentration of + 1 per cent. corresponds in this experiment with a decrease of the conductivity to the average amount of 8·1 per cent.; that is to say, the protracting effect of the colloid has slightly increased.

(2) *Globuline.*

The globulines precipitated by dilution, as above mentioned, are washed until the washing water shows a conductivity of $0 \cdot 2 \times 10^{-4}$. The deposit is dried and used for a 3 per cent. stock solution with $\frac{n}{2}$ Na OH. Mixture is kept in the ice-box for forty-eight hours before used for experiments.

13/12/07. T = 37°.

	9.30 a.m.	10 a.m.	12.30 p.m.	1 p.m.
Conductivity $\times 10^{-4}$ of 3 per cent. stock solution ..	863		888	
Conductivity $\times 10^{-4}$ of $\frac{n}{2}$ Na OH		1094		1093

The conductivity of globuline solution increased within three hours to the amount of 25 = about 3 per cent. The cause is very likely the high temperature of the room provoking alterations of the equilibrium between colloid particles and ions.

13/12/07. T=37°.

Concentration of Globuline. %	Conductivity × 10^{-4}.	Differences, absolute.	Difference in percentages. %
0 ·($\frac{n}{2}$ Na OH)	1094		
0 ·5	1057	37	3 ·4
1 ·0	1023	34	3 ·2
1 ·5	988	35	3 ·4
2 ·0	954	34	3 ·4
2 ·5	921	33	3 ·5
3 ·0	888	33	3 ·6

One per cent. increase in concentration of the colloid causes a decrease of the conductivity of the system amounting to 6 ·8 per cent.

The serum globuline decreases the conductivity somewhat less than albumine. The curve of the former is almost a straight line, whilst the curve of the latter is very slightly concave towards the abscissa.

It is not allowed to apply these results *tale quale* to serum, because

(1) the albumines and globulines are in another state than they are in $\frac{n}{2}$ Na OH solution, and therefore affect the conductivity in a different manner;

(2) the serum colloids are suspended in a mixed solution of various electrolytes and non-electrolytes, and the conductivity of such solutions of numerous components is quantitatively influenced by colloids in another manner than a simple solution. But we can safely say that the depression of conductivity of a serum which contains about 8 per cent. albuminoid substances is very great, that there is a certain normal proportion between the conductivity of the pure salt serum (deprived of colloids) and the conductivity of the native serum. This proportion will probably be another in pathological conditions according to alterations of colloids and electrolytes. At present we can only make the statement that the conductivity of serum is altered in diseases (compare tables later), especially in piroplasmosis, and that this phenomenon can be used as diagnosticum.

The following experiments with gelatine and saponine are analoga to the former with serum colloids regarding the diminution of conductivity :—

(3) *Gelatine.*

A 2 per cent. stock solution in $\frac{n}{2}$ Na OH is made on 3rd January, 1908, and kept in the ice-chest till 7th January, 1908.

7/1/08. T=37°.		Conductivity × 10^{-4}
2 per cent. Gelatine solution..	11.30 a.m.	980
	12 a.m.	980
	6 p.m.	973
	6.20 p.m.	972
$\frac{n}{2}$ Na OH	10.30 a.m.	1099
	6.40 p.m.	1100
8/1/08.		
2 per cent. Gelatine solution having been in ice-chest over-night	10.30 a.m.	954

The conductivity of the gelatine-alkali solution decreases, while the latter is kept at room temperature (30° C.) for seven hours, to the amount of 8×10^{-4} = about 0·8 per cent.; then a further decrease takes place when the solution is in the ice-box for sixteen hours, amounting to $18 \times 10^{-4} = 1{\cdot}85$ per cent. A similar decrease could be noted with the albumine.

7/1/08. T=37°.

Concentration of Gelatine. %	Conductivity × 10^{-4}.	Differences, absolute.	Difference in percentages. %
0·($\frac{n}{2}$ NaOH)	1099		
0·25	1082	17	1·5
0·50	1068	14	1·3
0·75	1054	14	1·3
1·0	1039	15	1·4
1·25	1025	14	1·3
1·50	1008	17	1·7
1·75	989	19	1·9
2·00	973	16	1·6

When the concentration of gelatine increases to the amount of 1 per cent. the conductivity of the system shows a decrease of 6 per cent.

(4) *Saponine.*

The conductivity of a 4 per cent. saponine solution in $\frac{n}{2}$ NaOH remains constant, adversely to globuline, albumine, and gelatine solutions. The 4 per cent. stock solution was made on the 3rd January, 1908, and kept at the same conditions as the gelatine solution.

	37°.	Conduct. × 10^{-4}
4 per cent. Saponine solution—7/1/08.—	11.15 a.m.	882
	11.45 a.m.	882
	6.10 p.m.	882
	6.30 p.m.	879
	8/1/08—10.45 a.m.	880

7/1/08. T=37°.

Concentration of Saponine. %	Conductivity × 10^{-4}.	Differences, absolute.	Difference in percentages. %
0·($\frac{n}{2}$ NaOH)	1096		
0·5	1065	31	2·8
1·0	1035	30	2·8
1·5	1007	28	2·7
2·0	979	28	2·8
2·5	953	26	2·7
3·0	928	25	2·6
3·5	903	25	2·7
4·0	881	22	2·4

Increase of saponine concentration of 1 per cent. provokes decrease of the conductivity of the system amounting to 5·4 per cent. Gelatine influences the resistance of the system almost as intensively as saponine, but both somewhat less than albumine and globuline.

(5) *Erythrocytes.*

A suspension of erythrocytes as a heterogeneous system is an obstacle for the migration of ions in the interglobular liquid.*

10/1/08. T=37° C.

Mixtures of various concentrations of defibrinated blood of normal horse 3250 and serum of the same animal:

Volume of blood corpuscles. %	Conductivity $\times 10^{-4}$ of the suspension.	Differences absolute.	Differences for 1% increase of the vol. of corp.	Difference % for 1 % volume of the corp. %
0 (Serum)	146·0			
13·3	117·9	28·1	2·1	1·5
17·8	107·1	10·8	2·4	2·0
22·2	98·5	8·6	2·0	1·9
26·7	89·0	9·5	2·1	2·1
31·1	79·4	9·6	2·2	2·5
35·6	68·9	10·5	2·3	2·9
40·0	57·6	11·3	2·6	3·8

Increase of the volume of blood corpuscles corresponds with continually increasing decrease of the conductivity.

This method could be used to find the volume of blood corpuscles in a system by means of the electrical resistance and subsequent interpolation on the curve.

E.—COMPARATIVE PHYSICAL-CHEMICAL RESEARCH ON HORSE BLOOD AND SERUM.

(With Special Reference to Horse-sickness.)

These experiments were undertaken to find differences by means of various physical methods combined between

(1) Normal horses;

(2) Horses suffering from horse-sickness;

(3) Horses immune and hyperimmune against horse-sickness;

(4) Serum horses, i.e. horses from which great quantities of blood have been taken (artificial anaemia).

(1) *Normal Horses.*

It is naturally necessary to know the normal values forming the basis with which results of pathological cases can be compared; first of all the variations undergone by the various values of the experiments on one and

* Also in suspensions of mineral particles (sand) a decrease of conductivity takes place.—*Oker-Blom*, Pflüger's Arch., *79*, 1900—*cit.* Hamburger, Osmot, Druck, etc.

the same horse at different days had to be ascertained. For this purpose blood and serum of two horses (3682 and 3685) were examined day by day for about five weeks. One hundred and fifty cubic centimetres of blood were taken at the time, that is to say, the same quantity which was drawn from the various horses during the experiments on piroplasmosis and horse-sickness. Thus the eventual effect of continual bleeding on blood and serum could be studied and at the same time the question answered as to whether this loss of blood does or does not influence the result of the investigations on piroplasmosis and horse-sickness.

The values from the examinations contained in the tables are of the following matters :—

(1) Temperature of the body.
(2) Volume of blood corpuscles.
(3) Specific gravity of blood.
(4) Viscosity of blood.
(5) Conductivity of serum.
(6) Specific gravity of serum.
(7) Viscosity of serum.
(8) Coefficient of optical refraction of serum.
(9) Daily amount of water drunk by the animal.

Horse 3682.

Date.	Temperature.		Blood.			Serum.					
	Morning. F.	Evening. F.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity 37° C.	Specific Gravity 37° C.	Conductivity at 37° × 10^{-4}	Viscosity at 25° C.	Drink-water.	Index of Refraction at 37° C.	Amount of Serum (Blood = 1)
June 24..	99·2	100·8	32	4·43	1·0506	1·0256	141·8	2·01	—	—	1/2
,, 25..	99·0	101·4	32	3·47	1·0499	1·0252	141·2	1·78	—	—	1/2
,, 26..	99·8	101·6	36½	3·67	1·0514	1·0266	144·5	1·82	—	—	1/2
,, 27..	100·0	101·4	31½	3·48	1·0507	1·0252	145·3	1·76	—	—	1/2
,, 28..	100·2	101·6	35½	—	1·0530	1·0263	148·4	1·82	—	—	1/2
,, 29..	99·0	102·2	39	3·87	1·0535	1·0257	145·3	1·78	—	1·34556	1/2
,, 30..	100·2	101·8	33½	3·36	1·0509	1·0253	150·1	—	—	—	1/2
July 1..	99·6	101·4	31	3·15	1·0479	1·0235	147·2	—	—	—	2/5
,, 2..	99·0	102·2	31½	3·24	1·0480	1·0243	145·3	—	—	—	1/2
,, 3..	99·0	101·4	31	3·54	1·0503	1·0249	144·4	—	—	—	1/2
,, 4..	99·6	101·4	33	—	1·0491	1·0249	145·1	—	—	—	1/2
,, 5..	99·6	101·0	33	—	1·0502	1·0254	146·6	—	—	—	1/2
,, 6..	98·4	101·4	34½	—	1·0520	1·0256	144·7	—	—	—	1/2
,, 7..	99·0	101·8	35½	—	1·0503	1·0253	144·4	—	—	1·34605	2/5
,, 8..	99·6	102·0	33	—	1·0493	1·0249	145·3	—	—	—	2/5
,, 9..	99·6	101·8	33	—	1·0492	1·0249	149·0	—	—	—	2/5
,, 10..	99·6	100·6	32½	—	1·0481	1·0239	147·0	1·68	—	1·34546	2/5
,, 11..	99·0	100·6	30	—	1·0473	1·0242	145·5	1·68	—	1·34539	3/5
,, 12..	99·0	100·6	29½	—	1·0489	1·0243	148·4	1·70	—	1·34559	1/2
,, 13..	100·0	100·8	29½	—	1·0479	1·0236	151·2	1·58	—	1·34499	1/2
,, 14..	—	—	28	3·80	1·0491	1·0256	150·0	1·79	—	1·34569	2/5
,, 15..	—	—	32½	3·87	1·0499	1·0250	151·0	1·68	—	1·34582	1/2
,, 16..	—	—	31½	3·62	1·0487	1·0244	153·8	1·57	10½+6	—	1/2
,, 17..	—	—	29	3·42	1·0466	1·0236	148·5	1·60	5½+1	—	1/2
,, 18..	—	—	31½	3·25	1·0472	1·0240	145·8	1·67	2½+1	—	1/2
,, 19..	—	—	34½	3·47	1·0475	1·0240	145·7	—	3½+3	—	2/5
,, 20..	—	—	36	—	1·0511	1·0252	143·3	—	2½+3	—	2/5

„ 21..	—	—	33½	—	1·0501	1·0252	142·3	—	1 +0	—	2 5
„ 22..	—	—	31½	—	1·0498	1·0252	145·4	—	2 +1	—	2 5
„ 23..	—	—	30	—	1·0480	1·0247	145·3	—	4 +2	—	1 2
„ 24..	—	—	33	—	1·0492	1·0254	146·5	—	3½+6½	—	1 2
„ 25..	—	—	30	—	1·0470	1·0249	145·4	—	3½+2½	—	1 2
„ 26..	—	—	30½	—	1·0440	1·0240	148·1	—	10 +3	—	1 2
„ 27..	—	—	27	—	1·0458	1·0240	146·5	—	No water given	—	1 2
„ 28..	—	—	29	—	1·0477	1·0252	151·0	—	11 +6½	—	1 2
„ 28..	—	—	29½	—	1·0430	1·0231	144·2	—	—	—	1 2
„ 29..	—	—	27½	—	1·0454	1·0243	143·7	—	3½+4	—	1 2

Horse 3685.

Date.	Temperature.		Blood.			Serum.					
	Morning. F.	Evening. F.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^{-4}	Viscosity at 25° C.	Drink-water.	Index of Refraction at 37° C.	Amount of Serum (Blood=1)
June 24..	99·0	100·0	35½	3·06	1·0500	1·0226	152·0	1·68	—	—	½
„ 25..	98·2	100·0	35	3·46	1·0505	1·0226	147·0	1·58	—	—	½
„ 26..	99·2	99·0	35½	3·56	1·0534	1·0240	153·0	1·63	—	—	½
„ 27..	98·4	99·4	34	3·09	1·0526	1·0236	150·1	1·61	—	—	½
„ 28..	99·0	98·6	34	3·87	1·0530	1·0237	153·2	1·60	—	—	⅓
„ 29..	98·4	100·0	35	3·36	1·0517	1·0232	149·4	1·60	—	—	½
„ 30..	99·2	100·6	36½	3·20	1·0538	1·0237	153·0	—	—	—	½
July 1..	98·4	100·0	33	2·88	1·0496	1·0227	148·9	—	—	—	⅖
„ 2..	98·0	100·0	33	3·13	1·0500	1·0231	147·1	—	—	—	½
„ 3..	97·0	100·6	37	3·49	1·0524	1·0235	147·7	—	—	—	⅖
„ 4..	99·2	99·0	38½	—	1·0550	1·0239	150·8	—	—	—	½
„ 5..	98·0	98·4	37	—	1·0538	1·0235	153·1	—	—	—	⅖
„ 6..	99·0	99·6	36	—	1·0532	1·0244	148·8	—	—	—	½
„ 7..	98·4	99·0	41½	—	1·0578	1·0248	154·1	—	—	1·34553	⅖
„ 8..	98·0	100·0	38½	—	1·0536	1·0236	151·5	—	—	—	⅖
„ 9..	98·4	100·0	39	—	1·0543	1·0239	152·9	—	—	—	⅖
„ 10..	98·0	99·0	39	—	1·0536	1·0234	149·5	1·60	—	1·34491	⅖
„ 11..	98·4	100·6	40½	—	1·0561	1·0237	148·4	1·65	—	1·34514	½
„ 12..	99·0	100·8	36½	—	1·0545	1·0235	147·7	1·59	—	1·34476	½
„ 13..	99·0	100·2	38½	—	1·0547	1·0235	147·9	1·59	—	1·34471	½
„ 14..	99·6	99·0	40½	4·40	1·0563	1·0246	150·3	1·68	—	1·34541	½
„ 15..	99·0	100·4	37½	3·93	1·0558	1·0241	152·0	1·59	—	1·34516	½
„ 16..	98·4	100·8	36½	—	1·0515	1·0226	148·2	1·49	12 +1	—	½
„ 17..	99·4	100·0	34	3·52	1·0512	1·0230	148·2	1·54	13 +0	—	½
„ 18..	99·0	100·0	38½	3·83	1·0523	1·0235	145·7	1·57	1 +0	—	½
„ 19..	99·0	100·4	41½	4·48	1·0551	1·0238	148·0	—	4 +1	—	½
„ 20..	98·6	100·2	39	—	1·0532	1·0239	146·0	—	1½+1	—	½

„ 21..	99·2	100·8	$38\frac{1}{2}$	—	1·0543	1·0248	146·8	—	$2\frac{1}{2}+8$	—	$\frac{1}{2}$
„ 22..	98·4	100·0	$36\frac{1}{2}$	—	1·0533	1·0239	149·4	—	$6\frac{1}{2}+2\frac{1}{2}$	—	$\frac{1}{2}$
„ 23..	98·4	100·6	$38\frac{1}{2}$	—	1·0523	1·0237	150·3	—	6 +4	—	$\frac{1}{2}$
„ 24..	98·4	99·6	$37\frac{1}{2}$	—	1·0530	1·0239	151·5	—	10 +4	—	$\frac{1}{2}$
„ 25..	99·4	99·0	34	—	1·0504	1·0232	151·2	—	12 +0	—	$\frac{1}{2}$
„ 26..	99·0	101·0	$33\frac{1}{2}$	—	1·0496	1·0232	151·8	—	9 +4	—	$\frac{1}{2}$
„ 27..	98·4	100·4	36	—	1·0520	1·0237	152·9	—	No water given.	—	$\frac{1}{2}$
„ 28..	99·6	100·8	$33\frac{1}{2}$	—	1·0507	1·0235	152·3	—	12 +3	—	$\frac{1}{2}$
„ 29..	99·0	—	$36\frac{1}{2}$	—	1·0488	1·0230	146·8	—	—	—	$\frac{1}{2}$
„ 30..	—	—	36	—	1·0512	1·0235	150·2	—	10 +5	—	$\frac{1}{2}$

Horse 3682, grey mare, fifteen years old, bad condition.

It is normal during twenty days; afterwards a fever sets in, probably a sign of a slight horse-sickness attack, for the volume of blood corpuscles increase.

Volume of blood corpuscles and specific gravity of blood are going parallel; that is to say, they increase and decrease at the same time, but the degree of variation is different, namely, for the volume about fifty times greater than of the specific gravity. Both, volume and specific gravity, show two periods of increase and decrease, so that the final values are somewhat lower than the incipient ones. (Compare piroplasmosis and horse-sickness.)

In face of this fact it has to be taken into consideration that in cases of serial experiments on piroplasmosis or horse-sickness on horses in poor condition the decrease of red blood corpuscles might partially be due to the daily bleedings, though comparatively small quantities were taken (150 c.c.).

The viscosity of blood, as far as it is examined, goes also up and down with the volume of red blood corpuscles, but the variations are wider.

Viscosity and specific gravity of serum, compared with one another, show similar behaviour as the respective values of the blood. Like the specific gravity of the blood that of the serum has two periods and is finally lower than originally.

The conductivity increases from the beginning to the end of the research period, and must, of course, behave divergently with the specific gravity.

The quantity of water taken by the animal depends on the conductivity of serum; the animal drinks more when the conductivity is high, less when it is low. (Water given after the animal has been bled.)

Horse 3685, chestnut gelding, ten years old, good condition.

Volume of blood corpuscles and specific gravity of blood, besides their approximative parallelism, show a slight and slow increase, then a decrease to their respective original values. In this animal, the perpetual loss of blood rather acts as a stimulus for reproduction and a slight superproduction of erythrocytes.

The viscosity of blood and serum behaves similarly as in 3682. The same is to be said about specific gravity and conductivity of serum. Both the latter show divergencies as in 3682, which very likely are due to the decrease of colloids or non-electrolytes.

There is again the same dependence of the amount of water taken by the animal on the conductivity of serum as an expression of the tendency of the organism to keep the electrolyt concentration constant.

Average values and variations of the results are shown by the following tables:—

Normal Horse 3682.

Horse 3682.	Temperature.		Blood.			Serum.			
	Morning. F.	Evening. F.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10⁻⁴	Viscosity at 25° C.	Index of Refraction at 37° C.
Number of examinations ..	20	20	20	10	20	20	20	10	8
„ days	20	20	20	10	20	20	20	10	8
Average	*99·4*	*101·4*	*32·8*	*3·60*	*1·0499*	*1·0250*	*146·0*	*1·76*	*1·34557*
Maximum	100·2	102·2	39·0	4·43	1·0535	1·0266	151·2	2·01	1·34605
Minimum	98·4	100·6	29·5	3·15	1·0473	1·0236	141·2	1·58	1·34499
Variation above average ..	0·8 %	0·8 %	19 %	23 %	0·34 %	0·16 %	3·6 %	14 %	0·04 %
„ below „ ..	1·0 %	0·8 %	10 %	12 %	0·25 %	0·14 %	3·3 %	10 %	0·04 %
„ total	1·8 %	1·6 %	29 %	35 %	0·59 %	0·30 %	6·9 %	24 %	0·08 %
Values above average	50 %	40 %	50 %	40 %	50 %	50 %	40 %	60 %	50 %
„ below „	50 %	60 %	50 %	60 %	50 %	50 %	60 %	40 %	50 %

Normal Horse 3685.

Horse 3685.	Temperature.		Blood.			Serum.			
	Morning. F.	Evening. F.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^{-4}	Viscosity at 25° C.	Index of Refraction at 37° C.
Number of examinations ..	36	35	36	15	36	36	36	15	7
„ days	36	35	36	15	36	36	36	15	7
Average	*98·7*	*99·9*	*36·9*	*3·50*	*1·0529*	*1·0236*	*149·9*	*1·60*	*1·34509*
Maximum	99·6	101·0	41·5	4·48	1·0578	1·0248	153·2	1·68	1·34553
Minimum	97·0	98·4	33	2·88	1·0488	1·0226	145·7	1·54	1·34471
Variation above average ..	0·9 %	1·1 %	12·5 %	28 %	0·46 %	0·12 %	2·2 %	5 %	0·03 %
„ below „ ..	1·7 %	1·5 %	10·5 %	18 %	0·39 %	0·10 %	2·8 %	4 %	0·03 %
„ total	2·6 %	2·6 %	23 %	46 %	0·85 %	0·22 %	5·0 %	9 %	0·06 %
Values above average	50 %	67 %	47 %	47 %	56 %	53 %	50 %	53 %	57 %
„ below „	50 %	33 %	53 %	53 %	44 %	47 %	50 %	47 %	43 %

The results of the various methods arranged in descending order of their variations give the following series for both horses :—

Viscosity blood, vol. blood corp., viscosity serum, conductivity, temperature, specific gravity blood, specific gravity serum, index of refraction.

The following values are higher :—

In 3682 : *Temperature, viscosity of blood, specific gravity serum, viscosity serum, index of refraction.*

In 3685 : *Vol. blood corp., specific gravity blood, conductivity*

The variations are greater :—

In 3682 : *Vol. blood corp., specific gravity serum, conductivity, viscosity serum, index of refraction.*

In 3685 : *Temperature, viscosity blood, specific gravity blood.*

That is to say : In three instances (temperature, vol. blood corp., viscosity blood) the lower values show greater variations.

Conclusions.

The number of blood corpuscles and, of course, the specific gravity of blood, increase in a horse which is in good condition, and decrease in a horse of bad condition when a loss of blood amounting to 150 c.c. takes place every day. (Daily withdrawing of blood, as it is necessary in serial experiments, therefore might have a slight influence on the result when a horse is in a poor condition.)

The viscosities of blood and serum increase or decrease as a rule with the respective specific gravities.

The quantity of water taken by the animals depends (among other factors) on the conductivity of serum, and, if the latter is high, the former is great and vice versa.

* * *

In the following table the results of the application of five different physical-chemical methods on the study of blood and serum of fifty different horses are contained. The last column shows the quantities of serum (in comparison to the entire quantity drawn from an animal) obtained twenty-four hours after tapping. The blood was kept in a cool room, where it coagulated, and the fibrin clot contracted itself.

Date.	Number.	Sex.	Age.	Condition.	Blood.			Serum.				
					Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^{-4}	Viscosity at 25° C.	Surface Tension at 37° C.	Amount of Serum (Blood=1)
11/6/08	3618	Gelding	13 years	—	28	—	1·0468	1·0235	147·4	—	—	$^{2}/_{5}$
11/6/08	3619	,,	10 ,,	—	37	4·47	1·0558	1·0270	140·5	1·90	—	$^{1}/_{5}$
15/6/08	3623	,,	16 ,,	—	36½	3·63	1·0530	1·0251	146·4	1·81	5·98	$^{1}/_{2}$
15/6/08	3625	,,	7 ,,	—	31	3·73	1·0504	1·0239	150·3	1·64	5·78	$^{1}/_{2}$
15/6/08	3629	Mare	7 ,,	—	31½	3·61	1·0498	1·0257	149·5	1·82	—	$^{1}/_{2}$
15/6/08	3630	,,	12 ,,	—	39	4·29	1·0560	1·0257	148·4	1·93	6·11	$^{1}/_{2}$
15/6/08	3631	,,	12 ,,	—	34	3·98	1·0528	1·0248	147·6	1·74	—	$^{1}/_{2}$
15/6/08	3632	Gelding	14 ,,	—	32½	3·90	1·0525	1·0274	146·7	1·87	5·70	$^{1}/_{2}$
15/6/08	3635	,,	10 ,,	—	35½	4·00	1·0512	1·0243	146·1	1·70	6·16	$^{1}/_{2}$
15/6/08	3636	,,	13 ,,	—	33	4·05	1·0545	1·0275	146·5	1·94	6·06	$^{1}/_{2}$
23/6/08	3637	,,	8 ,,	Very good	35½	4·59	1·0532	1·0274	142·3	2·04	—	$^{2}/_{5}$
23/6/08	3638	,,	Aged	Fairly good	27	3·86	1·0488	1·0265	141·6	1·95	—	$^{1}/_{2}$
19/6/08	3639	,,	,,	Poor	32½	—	1·0526	1·0253	144·3	1·84	—	$^{1}/_{2}$
19/6/08	3641	,,	8 years	Good	38	4·15	1·0545	1·0263	144·0	1·66	—	$^{2}/_{5}$
19/6/08	3642	,,	Aged	,,	42	5·27	1·0586	1·0272	142·4	1·91	—	$^{2}/_{5}$
19/6/08	3643	,,	,,	Fairly good	30	3·61	1·0501	1·0255	144·2	1·72	—	$^{1}/_{5}$
19/6/08	3644	Mare	,,	Good	29½	3·97	1·0488	1·0249	142·4	1·72	—	$^{1}/_{3}$
19/6/08	3645	Gelding	10 years	Rather poor	29	3·42	1·0472	1·0236	144·2	1·71	—	$^{1}/_{2}$
23/6/08	3646	,,	12 ,,	Very good	29	3·37	1·0498	1·0265	148·2	1·84	—	$^{1}/_{2}$
23/6/08	3647	,,	Aged	Rather poor	33	3·97	1·0503	1·0252	147·6	1·82	—	$^{1}/_{2}$
22/6/08	3662	,,	,,	,,	39	3·92	1·0547	1·0263	145·4	1·74	—	$^{1}/_{2}$
22/6/08	3663	,,	13 years	Good	35	4·15	1·0508	1·0245	150·0	1·67	5·37	$^{1}/_{3}$
22/6/08	3664	,,	Aged	Fairly good	33½	3·16	1·0497	1·0255	141·9	1·74	—	$^{1}/_{2}$
22/6/08	3665	,,	10 years	Rather poor	36½	3·49	1·0508	1·0265	142·5	1·80	—	$^{1}/_{2}$

22/6/08	3666	,,	17 years	Very good	40	4·11	1·0547	1·0265	147·2	1·75	—	1/2
22/6/08	3667	,,	Aged	Good	38½	4·49	1·0567	1·0260	144·6	1·76	—	1/2
22/6/08	3668	,,	17 years	Fairly good	33	3·45	1·0498	1·0251	147·6	1·65	5·89	1/2
22/6/08	3669	,,	13 ,,	Good	37	3·63	1·0510	1·0255	142·1	1·80	—	2/5
22/6/08	3670	,,	Aged	Fairly good	30	3·71	1·0495	1·0278	143·7	1·99	5·85	1/2
24/6/08	3675	,,	13 years	Rather poor	27	3·64	1·0474	1·0271	141·5	2·09	5·87	2/5
24/6/08	3679	,,	Aged	,,	32	3·09	1·0498	1·0263	144·2	1·87	—	2/5
24/6/08	3680	,,	,,	,,	28½	2·91	1·0447	1·0229	146·4	1·69	—	1/2
24/6/08	3681	,,	20 years	,,	29½	3·11	1·0478	1·0249	140·8	1·79	—	1/2
24/6/08	3682	Mare	12 ,,	,,	32	4·33	1·0506	1·0256	141·8	1·73	6·00	1/2
24/6/08	3683	Gelding	Aged	Fairly good	31	3·28	1·0506	1·0251	145·0	1·79	—	1/2
24/6/08	3684	,,	17 years	Rather poor	31	3·40	1·0487	1·0244	143·9	2·01	—	1/2
24/6/08	3685	,,	13 ,,	Good	35½	3·06	1·0500	1·0226	152·0	1·68	5·49	1/2
26/6/08	3701	,,	10 ,,	,,	32½	4·21	1·0534	1·0306	145·7	2·13	—	2/5
26/6/08	3702	,,	13 ,,	Fairly good	31½	3·28	1·0503	1·0272	148·2	1·79	—	1/2
26/6/08	3703	,,	15 ,,	Good	37½	3·67	1·0538	1·0256	148·3	1·79	—	1/2
26/6/08	3704	,,	16 ,,	Fairly good	32½	3·71	1·0489	1·0253	143·4	1·74	—	1/2
16/1/08	3253	—	—	Very good	34	—	1·0566	1·0297	155·7	—	—	—
16/1/08	3260	—	—	,,	37	—	1·0594	1·0282	157·6	—	—	—
16/1/08	3248	—	—	,,	39	—	1·0578	1·0280	156·9	—	—	—
16/1/08	3249	—	—	,,	37	—	1·0605	1·0290	156·5	—	—	—
16/1/08	3256	—	—	,,	34	—	1·0540	1·0254	160·4	—	—	—
16/1/08	3261	—	—	,,	39	—	1·0577	1·0264	157·5	—	—	—
7/10/07	2917	—	—	—	31	—	—	1·0268	146·4	—	—	—
6/10/07	2915	—	—	—	39	—	—	1·0283	143·8	—	—	—
12/2/08	2904	—	—	—	35	—	1·0528	1·0270	148·3	—	—	—

The average values are collected in the next table, which also shows the degrees of variations and the limits within which the physical-chemical values of different horses vary.

NORMAL HORSES—VARIATIONS.

	Blood.		Serum.				
	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at $37° \times 10^{-4}$.	Viscosity at 25° C.	Surface Tension at 37° C.
Number of examinations	100 *	90 *	48	50	50	81 *	42 *
,, animals	100	72	48	50	50	71	36
Average values	33·4	3·80	1·0521	1·0261	146·8	1·83	5·95
Maximum	43	5·27	1·0605	1·0306	160·4	2·13	6·45
Minimum	22	2·95	1·0447	1·0226	140·5	1·55	5·37
Total variation	63 %	61·1 %	1·50 %	0·78 %	13·5 %	31·8 %	18·2 %
Variation above average ..	29 %	38·7 %	0·80 %	0·44 %	9·2 %	16·7 %	8·4 %
,, below ,, ..	34 %	22·4 %	0·70 %	0·34 %	4·3 %	18·1 %	9·8 %
Percentage of values above average ..	48 %	41·1 %	46 %	48 %	40 %	37 %	57 %
,, ,, below ,,	52 %	58·9 %	54 %	52 %	60 %	63 %	43 %

* Besides the above written, results of other experiments are taken into calculation.

The particularities of blood and serum, arranged in descending order of the latitudes of their variations, are:

Vol. blood corp., viscosity blood, viscosity serum, surface tension serum, conductivity, specific gravity blood, specific gravity serum.

Horses 3682 and 3685 gave the same order of the serum values; the order of vol. blood corp. and viscosity of blood, however, is reversed.

The specific gravity of the blood is chiefly dependent on the volume of corpuscles, that is to say, when a blood is rich in globules, it can be expected that its specific gravity is high.

The viscosity is influenced by both, but in a series of horses the latter follows more the specific gravity than the volume of corpuscles, and only from the viscosity 4·0 upwards it is allowed to say: the higher the internal friction of the blood the greater its specific gravity (above 1·050) and the number of corpuscles.

In numerous other experiments, however, there is a much closer relation between viscosity of blood and volume percentage of globules. (See chapter on Viscosity.) As the viscosity of serum is mainly due to the colloids and the latter protract the electric conductivity, a relation between these two values had to be expected. As a matter of fact in the majority of instances a high viscosity corresponds with a low conductivity; but the product conductivity × viscosity does not show a stability within the limits of errors. Our experiments do not allow to bring forward a mathematical formula as the expression of the relation between internal friction and conductivity as it was found for pure and simple electrolytic solutions.* The same is to be said about the relation between internal friction and specific gravity. Though in most of the examined sera, the specific gravity is high when the viscosity is considerable, the quotient $\frac{\text{viscosity}}{\text{specific gravity}}$ is not constant, and only in a little more than half of the cases it is possible to calculate one from the other satisfactorily.

The few values of surface tension allow not to draw definite conclusions with regard to mathematical relations with other physical properties of the serum; apparently there are none, and the surface tension seems to stand by itself.

* *Pissarjewski and Karp*, Zeitsche. Physik. Chemie. *63*, 257, 1908.

Horse-Sickness, Beginning.

Date.	Number.	Sex.	Age.	Condition	Temperature.		Blood.			Serum.			
					Morning. F.	Evening. F.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^{-4}	Viscosity at 25° C.	Amount of Serum (Blood=1)
11/6/08	3302	Gelding	8 yrs.	—	102·0	104·2	25	3·26	1·0451	1·0256	142·4	1·79	$^2/_5$
24/6/08	3400	,,	10 ,,	Fairly good	101·6	103·4	29	—	1·0490	1·0270	140·5	—	$^2/_5$
25/6/08	3450	,,	11 ,,	Good	100·6	105·8	31	3·58	1·0487	1·0256	141·1	—	$^1/_3$
25/6/08	3630	Mare	11 ,,	Very good	100·2	103·2	38	3·84	1·0529	1·0246	144·6	1·76	$^2/_5$
17/10/07	2915	—	—	—	101·0	99·4	38	—	—	1·0282	148·8	—	—
15/10/07	2917	—	—	—	103·0	103·2	27	—	—	1·0265	149·8	—	—
25/2/08	3091	—	—	—	101·8	104·0	34	—	—	1·0279	142·9	—	—

(2) Horses Suffering from Horse-Sickness.

Horses suffering from horse-sickness have lower average values with all methods applied, only the average volume of blood corpuscles is a little higher than in normal horses. During the climax of the disease also the viscosity is supernormal besides the volume. The relative blood stasis in the jugular vein, accompanied by accumulation of CO_2 in highly sick animals, explains this phenomenon. As the majority of simply sick animals (66 per cent.) has a subnormal blood viscosity (and volume percentage of globules) it is to be understood that the average can be lower than the normal average. The variations of both series of values are excessive, viz., of the volume of globules in nearly double, of the viscosity in more than double the latitudes under normal circumstances. (Compare chapter on Viscosity.) Similar reflections hold good for the specific gravity of blood.

While the maxima of volume of erythrocytes and maxima and minima of viscosity and specific gravity of *blood* exceed the respective normal limits, the highest pathological *serum* values never reach the supreme top elevations, but the minima of diseased serum go beyond the normal minima.

Date.	Number.	Sex.	Age.	Condition.	Temperature.		Blood.			Serum.					Day of Disease.	Clinical Observations.	Pulse.
					Morning.	Evening.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° C. × 10^{-3}	Viscosity at 25° C.	Surface Tension at 37° C.	Amount of Serum (Blood = 1).			
9/6/'08	3522	Gelding	8 yrs.	—	103·0	102·2	30	—	1·0448	1·0248	138·3	1·78	—	2/5	11	—	52
9/6/'08	3586	,,	Aged	—	101·6	103·0	28	—	1·0468	1·0237	139·9	1·76	—	2/5	11	—	46
10/6/'08	3572	,,	,,	—	102·2	104·8	27	—	10·446	1·0226	143·7	1·69	6·16	2/5	12	—	44
10/6/'08	3362	,,	12 yrs.	—	101·2	103·4	25	3·14	1·0452	1·0253	139·8	1·82	6·40	1/2	12	—	48
10/6/'08	3583	,,	7 ,,	—	101·8	102·6	34	—	1·0506	1·0255	141·8	1·96	6·35	2/5	12	—	40
10/6/'08	3570	,,	10 ,,	—	104·6	105·8	30	3·33	1·0459	1·0243	143·1	1·80	6·25	2/5	12	—	42
10/6/'08	3586	,,	Aged	—	100·6	99·0	39	4·44	1·0522	1·0221	141·1	1·61	—	1/5	12	Dikkop	46
10/6/'08	3571	,,	16 yrs.	—	102·8	103·8	29	3·45	1·0467	1·0236	142·7	1·68	6·40	3/5	10	—	44
11/6/'08	3582	,,	Aged	—	102·0	103·0	29	3·71	1·0481	1·0234	146·4	—	—	1/5	13	Dikkop	44
11/6/'08	3587	,,	,,	—	102·2	103·8	35	3·62	1·0485	1·0231	141·2	1·76	—	1/3	10	,, ? Just before death	50
11/6/'08	3572	,,	,,	—	102·2	103·6	30	3·54	1·0456	1·0208	143·1	1·65	—	2/5	13	Dikkop	48
11/6/'08	3362	,,	12 yrs.	—	101·4	102·4	25	—	1·0451	1·0252	142·9	1·89	—	2/5	13	—	46
12/6/'08	3572	,,	Aged	—	101·6	100·0	38	—	1·0506	1·0215	140·3	1·69	—	1/3	14	Dikkop; died next day	46
12/6/'08	3605	,,	,,	—	103·2	104·2	37½	—	1·0512	1·0234	138·1	1·81	—	2/5	9	—	54
12/6/'08	3302	,,	8 yrs.	—	103·2	105·8	25	3·05	1·0443	1·0246	140·1	1·79	5·91	2/5	14	—	80
12/6/'08	3519	,,	Aged	—	104·0	105·6	29	3·75	1·0483	1·0259	136·6	2·03	—	2/5	9	—	54
13/6/'08	3250	,,	10 yrs.	—	103·0	104·8	34	3·82	1·0501	1·0234	142·8	1·74	—	2/5	10	—	42
13/6/'08	3570	,,	10 ,,	—	104·0	101·6	44	4·55	1·537	1·0210	142·1	1·71	—	2/5	15	—	52
13/6/'08	3557	,,	7 ,,	—	102·0	104·8	26	3·39	1·0456	1·0251	139·7	1·82	—	1/2	15	—	60
16/6/'08	3591	,,	10 ,,	—	101·6	97·0	58	7·39	1·0655	1·0221	144·0	1·67	—	1/10*	10	Died next night	72
16/6/'08	3590	,,	13 ,,	—	102·0	105·4	30½	—	1·0484	1·0257	138·5	1·91	—	3/5	10	—	32
16/6/'08	3616	,,	16 ,,	—	103·0	104·0	38½	—	1·0497	1·0219	139 7	1·63	—	2/5	11	—	72
16/6/'08	3547	,,	16 ,,	—	101·8	103·0	40	4·51	1·0510	1·0212	139·8	1·64	—	2/5	11	Died next night	52
16/6/'08	3607	Stallion	14 ,,	—	102·2	103·0	32	3·79	1·0463	1·0247	146·8	1·75	—	2/5	11	Died next day	—
16/6/'08	3250	Gelding	10 ,,	—	103·2	103·4	50	—	1·0589	1·0200	137·6	—	—	1/10	13	Dikkop	70
19/6/'08	3254	,,	6 ,,	Exceed. poor	103·2	104·6	36	5·48	1·0540	1·0254	148·2	1·92	—	1/5	8	—	78
19/6/'08	3480	,,	11 ,,	Fairly good	102·0	102·0	41	4·15	1·0498	1·0204	137·8	1·58	—	1/5	16	—	78

19/6/08	3608	,,	8 ,,	Fairly good	105·0	105·2	38½	3·87	1·0498	1·0218	139·6	1·54	—	1/5 *	16	Dikkop	66
19/6/08	3372	,,	8 ,,	Very good	103·2	103·6	36½	4·54	1·0531	1·0260	137·7	1·83	—	1/5 †	5	Hyper.	54
19/6/08	3199	,,	8 ,,	Rather poor	103·0	102·8	33	5·03	1·0552	1·0284	130·9	2·07	—	1/5 *	6	—	66
20/6/08	3408	,,	Aged	Very good	104·2	103·6	38½	4·80	1·0541	1·0243	136·2	—	—	1/3	—	Died next day. Hyper. ‖	—
20/6/08	3306	,,	9 yrs.	Rather poor	103·2	103·0	25	3·89	1·0474	1·0270	144·6	—	—	2/5	6	—	—
20/6/08	3608	,,	8 ,,	Fairly good	103·0	100·4	49	4·37	1·0549	1·0198	138·5	1·54	5·67	1/10	17	Dikkop : died next night	70
20/6/08	3479	,,	10 ,,	Good	102·6	103·0	26	3·22	1·0439	1·0234	144·2	—	—	2/5	30	—	—
20/6/08	3411	,,	10 ,,	Very poor	103·4	100·0	53½	7·39	1·0652	1·0238	142·4	1·84	4·98	1/5 ‡	3	Died next night Hyper. ‖	—
20/6/08	3372	,,	8 ,,	Very good	103·2	103·2	37	5·23	1·0546	1·0256	141·2	1·99	5·10	1/5 †	6	Hyper. ‖ Urine dark	—
24/6/08	3457	,,	10 ,,	,,	105·8	104·6	29	3·09	1·0461	1·0234	141·5	1·84	5·97	2/5	5	—	—
24/6/08	3517	,,	13 ,,	Rather poor	102·2	105·0	32½	3·39	1·0469	1·0252	143·5	1·80	5·69	2/5	9	—	38
24/6/08	3445	,,	8 ,,	Good	103·0	105·4	27½	3·39	1·0475	1·0252	141·8	2·02	5·97	2/5	9	—	36
25/6/08	3457	,,	10 ,,	Very good	105·0	105·8	33	3·86	1·0474	1·0234	141·1	1·70	—	1/3 §	6	—	44
25/6/08	3338	,,	12 ,,	Rather poor	102 4	105·0	34½	3·75	1·0522	1·0247	143·7	1·75	—	1/2	6	—	—
25/6/08	3539	,,	13 ,,	Good	103·0	105·0	29	3·37	1·0468	1·0239	142·9	1·69	—	1/2	6	—	—
25/6/08	3627	Mare	9 ,,	Fairly good	104·0	103·8	30½	3·36	1·0476	1·0244	142·1	—	—	2/5	10	—	46
25/6/08	3631	,,	12 ,,	Good	102·0	104·8	32½	—	1·0503	1·0243	141·8	—	—	2/5	10	—	44
25/6/08	3635	Gelding	10 ,,	Fairly good	102·2	104·6	36½	3·83	1·0503	1·0234	143·2	1·72	—	1/3	10	—	30
29/6/08	3539	,,	13 ,,	,,	101·8	102·8	33½	3·62	1·0500	1·0209	142·0	1·58	—	2/5	10	—	—
30/6/08	3630	Mare	11 ,,	Good	105·4	105·0	38½	3·46	1·0528	1·0232	149·0	—	—	1/3	15	—	72
2/7/08	3675	Gelding	13 ,,	Rather poor	101·8	—	30	3·01	1·0459	1·0225	141·3	—	—	1/5	5	Died during the day	—
2/7/08	3710	,,	12 ,,	Good	101·8	104·0	31	3·44	1·0498	1·0254	140·5	—	—	1/3	6	—	46
3/7/08	3356	,,	11 ,,	,,	100·8	103·0	30½	2·92	1·0479	1·0248	146·0	—	—	1/10	18	—	46
3/7/08	3702	,,	13 ,,	Rather poor	100·2	103·6	32	3·35	1·0507	1·0270	144·2	—	—	2/5	7	—	—
3/7/08	3340	,,	7 ,,	Fairly good	101·0	101·6	23½	3·28	1·0443	1·0255	143·3	—	—	2/5	3 ?	—	—
3/7/08	3704	,,	16 ,,	,,	102·0	103·6	30	2·69	1·0469	1·0237	140·9	—	—	2/5	7	—	26
3/7/08	3630	Mare	11 ,,	Good	103·0	105·4	52	4·76	1·0604	1·0179	147·6	—	—	1/5	18	—	82
10/7/08	3662	Gelding	Aged	Rather poor	99·8	99·6	47	—	1·0541	1·0191	135·1	—	—	1/10	14	Dikkop : died next night	50
20/10/07	2915	—	—	—	103·2	103·4	36	—	—	1·0260	144·1	—	—	—	12	—	—
29/10/07	2917	—	—	—	104·0	105·0	27	—	—	1·0242	145·1	—	—	—	11	—	—
8/9/07	2975	—	—	—	104·6	106·4	27	—	—	1·0265	141·2	—	—	?	?	—	52
8/9/07	2961	—	—	—	—	—	45	—	—	1·0244	138·9	—	—	—	?	Dikkop : died during the day	—
28/2/08	3091	—	—	—	103·0	104·0	31	—	—	1·0279	147·4	—	—	—	15	—	—

* Coagulated. † Red brown. ‡ Reddish. § Orange. ‖ Attack of Horse-sickness following Hyperimmunisation.

Date.	Number.	Sex.	Age.	Condition.	Temperature.		Blood.			Serum.					Number of days from the date of infection.	Clinical Observations.	Pulse.
					Morning.	Evening.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10⁴	Viscosity at 25° C.	Index of Refraction at 37° C.	Amount of Serum (Blood = 1).			
10/6/08	3569	Gelding	15 yrs.	—	104·0	102·4	28	3·90	1·0473	1·0251	137·9	—	—	1/3	12	—	50
11/6/08	3522	,,	8 ,,	—	99·8	101·0	28	3·28	1·0460	1·0232	146·3	1·69	—	1/5	13	Dikkop	46
12/6/08	3582	,,	Aged	—	101·2	100·0	33½	3·67	1·0477	1·0216	142·9	—	—	1/5*	14	,,	48
12/6/08	3584	,,	6 yrs.	—	101·2	101·2	40½	5·28	1·0572	1·0300	140·5	2·37	—	1/3	8	—	—
12/6/08	*3522*	,,	8 ,,	—	100·0	101·0	29⅓	3·46	1·0469	1·0237	143·9	1·68	—	2/5	14	Dikkop	44
12/6/08	3571	,,	16 ,,	—	101·2	100·6	38	3·46	1·0493	1·0210	146·0	1·66	—	2/5	12	,,	—
13/6/08	3362	,,	12 ,,	—	99·4	104·0	26½	3·60	1·0444	1·0238	150·3	1·75	—	1/3	15	—	44
16/6/08	3302	,,	8 ,,	—	102·0	102·8	(31)	—	1·0444	1·0218	145·5	—	—	1/5	18	—	50
29/6/08	3459	,,	10 ,,	Good	100·4	101·2	36	3·12	1·0473	1·0190	137·5	—	—	1/10†	10	Dikkop	74
29/6/08	3631	Mare	12 ,,	,,	100·4	100·0	42	3·88	1·0546	1·0199	141·7	1·56	1·34226	1/5	14	—	50
29/6/08	3635	Gelding	10 ,,	Fairly good	99·0	101·0	46	4·76	1·0570	1·0200	140·3	1·53	—	1/3	14	Dikkop; died next night	36
29/608	3338	,,	12 ,,	,,	100·6	101·0	31	3·26	1·0478	1·0228	142·1	1·70	1·34419	1/3	10	—	—
30/6/08	3445	,,	8 ,,	Good	100·0	100·8	37	3·18	1·0492	1·0208	140·8	—	—	1/3	15	Dikkop	34
30/6/08	3400	,,	10 ,,	Fairly good	102·0	101·4	38	—	1·0479	1·0189	139·6	—	—	2/5	15	,,	70
1/7/08	3539	,,	13 ,,	Good	99·0	100·8	30½	—	1·0459	1·0207	144·0	—	—	1/3	12	,,	—
2/7/08	3465	,,	6 ,,	Fairly good	99·0	102·2	23	2·31	1·0412	1·0223	149·5	—	—	2/5	13	—	—
2/7/08	3457	,,	10 ,,	Very good	101·0	101·8	27½	2·93	1·0415	1·0201	135·7	—	1·34423	1/3‡	13	Dikkop disap.	50
3/7/08	3634	Stallion	8 ,,	,,	100·8	100·4	42½	4·02	1·0559	1·0232	146·7	—	—	1/2	18	,,	30
3/7/08	*3400*	Gelding	10 ,,	Fairly good	102·0	103·8	(25½)	2·70	(1·0415)	1·0208	136·5	—	—	2/5‡	18	,,	78
3/7/08	*3445*	,,	8 ,,	Good	100·2	100·6	(28¼)	2·81	1·0459	1·0225	148·9	—	1·346538	2/5	18	,,	—
8/7/08	3340	,,	7 ,,	Fairly good	101·4	100·2	23½	—	1·0450	1·0257	141·8	—	—	2/5	8	—	—
8/7/08	3702	,,	13 ,,	Rather poor	101·2	101·8	33½	—	1·0503	1·0237	147·7	—	—	2/5	12	Dikkop ?	28
8/7/08	3704	,,	16 ,,	Fairly good	101·6	102·0	34	—	1·0485	1·0211	144·0	—	—	1/3	12	,, ,,	32
10/7/08	*3702*	,,	13 ,,	,,	100·6	101·6	37	—	1·0514	1·0212	147·4	1·58	1·34358	2/5	14	,, ,,	38
10/7/08	*3704*	,,	16 ,,	Rather poor	99·0	100·2	35½	—	1·0476	1·0193	144·7	1·49	1·34213	2/5	14	,, ,,	34
10/7/08	3705	,,	Aged	Good	99·8	101·0	36⅔	—	1·0503	1·0198	140·8	1·63	1·34366	2/5	14	,, ,,	64
10/7/08	3706	,,	18 ,,	Fairly good	99·2	100·4	36½	—	1·0484	1·0203	145·7	1·62	1·34308	2/5	14	,, ,,	32
10/7/08	3707	,,	11 ,,	Rather poor	101·6	101·0	26	—	1·0445	1·0239	141·3	1·79	1·34529	1/2	14	—	48
10/7/08	3667	,,	Aged	Good	99·0	99·0	53	—	1·0615	1·0193	138·1	—	1·34324	1/10†	14	Dikkop	60
10/7/08	3668	,,	17 yrs.	,,	99·2	99·2	37	—	1·0509	1·0209	138·5	—	1·34395	1/5	14	,,	48
10/7/08	3663	,,	13 ,,	,,	101·2	100·0	32	—	1·0454	1·0191	139·5	—	1·34301	1/5	14	,,	56
13/7/08	*3704*	,,	16 ,,	Fairly good	101·0	100·8	25	3·14	1·0426	1·0218	144·3	1·67	1·34374	2/5	17	,,	42
14/7/08	(*3706*)	,,	18 ,,	,,	99·0	Anus open	(27)	3·28	—	1·0217	147·7	1·68	1·34372	2/5	18	,, disap.	—

* Coagulated. † Orange. ‡ Opalescent. § Second half of temperature reaction.

Passed through Horse-Sickness Attack.

Date.	Number.	Sex.	Age.	Condition.	Temperature.		Blood.			Serum.					Number of days from the date of Infection.	Clinical Observations.	Pulse.
					Morning.	Evening.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37 × 10⁻⁹	Viscosity at 25° C.	Index of Refraction at 37° C.	Amount of Serum (Blood = 1).			
9/6/08	3579	Gelding	8 yrs.	—	Norm.	Norm.	29	—	1·0485	1·0267	137·8	1·96	—	1/3	11	—	48
13/6/08	3522	,,	8 ,,	—	,,	,,	28	3·48	1·0468	1·0248	143·0	1·86	—	1/2	15	Dikkop disap.	42
25/6/08	3306	,,	9 ,,	Fairly good	,,	,,	27½	3·72	1·0474	1·0264	139·9	1·96	—	2/5	11	—	—
29/6/08	3450	,,	11 ,,	Good	,,	,,	27½	2·95	—	1·0245	146·2	1·96	1·34481	1/5	10	—	—
1/7/08	3338	,,	12 ,,	Rather poor	,,	,,	33⅓	3·16	1·0499	1·0231	149·4	—	—	2/5	12	—	—
1/7/08	*3450*	,,	11 ,,	Good	,,	,,	33⅓	—	1·0500	1·0256	148·4	—	—	2/5	12	—	—
2/7/08	3487	,,	12 ,,	Fairly good	,,	,,	40½	4·19	1·0553	1·0228	150·0	—	—	2/5	13	—	—
2/7/08	3449	,,	Aged	Exceed. poor	,,	,,	28½	2·97	1·0453	1·0210	149·8	—	—	1/3	13	—	—
2/7/08	3475	,,	6 yrs.	Good	,,	,,	27	2·86	1·0457	1·0242	147·8	—	1·34456	2/5	13	—	—
3/7/08	3679	,,	Aged	Rather poor	,,	,,	29	3·00	1·0466	1·0253	141·1	—	—	2/5	7	—	—
8/7/08	3356	,,	11 yrs.	Good	,,	,,	28	—	1·0469	1·0243	142·6	—	—	1/5	23	—	—
10/7/08	3701	,,	10 ,,	,,	,,	,,	32	—	1·0496	1·0259	147·0	1·85	1·34689	2/5	14	—	36
13/7/08	3663	,,	13 ,,	,,	,,	,,	28	3·23	1·0440	1·0218	148·0	1·60	1·34311	2/5	17	Dikkop	42
13/7/08	3668	,,	17 ,,	,,	,,	,,	31	3·85	1·0484	1·0232	140·7	1·69	1·34473	2/5	—	—	—
16/6/08	*3522*	Gelding	8 yrs.	—	Norm.	Norm.	27½	3·55	1·0472	1·0256	146·2	1·83	—	2/5	18	—	—
16/6/08	3582	,,	Aged	—	,,	,,	19½	—	1·0377	1·0220	141·7	1·69	—	2/5	18	Dikkop disap.	—
25/6/08	3372	,,	8 yrs.	Good	,,	,,	32	3·25	1·0444	1·0200	128·8	1·58	—	1/5	11	,, ,,	—
29/6/08	3627	Mare	9 ,,	Rather poor	,,	,,	31	3·08	1·0488	1·0249	148·0	1·69	—	2/5	14	—	—
30/6/08	3625	Gelding	7 ,,	Fairly good	,,	,,	31	—	1·0508	1·0249	149·9	—	—	1/2	15	—	—
2/7/08	*3627*	Mare	9 ,,	,,	,,	,,	27	3·00	1·0470	1·0243	149·0	—	1·34473	2/5	17	—	—
8/7/08	3634	Stallion	8 ,,	Very good	,,	,,	42	—	1·0551	1·0243	150·9	—	1·34576	1/3	23	—	—
8/7/08	*3449*	Gelding	Aged	Exceed. poor	,,	,,	28½	—	1·0451	1·0226	150·0	—	—	2/5	19	—	—
8/7/08	3465	,,	6 yrs.	Rather poor	,,	,,	24	—	1·0456	1·0259	146·0	—	1·34642	2/5	19	—	—
10/7/08	3400	,,	10 ,,	Fairly good	,,	,,	24	—	1·0460	1·0274	140·3	2·06	1·34784	2/5	25	Dikkop disap.	—
13/7/08	*3701*	,,	10 ,,	Good	,,	,,	31	3·98	1·0490	1·0274	138·4	1·99	1·34743	2/5	17	—	40

Horse-Sickness—Examinations on one and the same Animal on Different Days.

Date.	Number.	Sex.	Age.	Condition.	Temperature.		Blood.			Serum.					Day of Disease.	State of Sickness.	Clinical Observations.	Pulse.
					Morning.	Evening.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^{-4}	Viscosity at 25° C.	Index of Refraction at 37° C.	Amount of Serum (Blood = 1).				
26/6/08	3702	Geld.	13 yrs.	Fairly good	Norm.	Norm.	31½	3·28	1·0503	1·0272	148·2	1·79	—	1/3	—	Before inj.	—	—
3/7/08	,,	,,	,,	Rather poor	100·2	103·6	32	3·35	1·0507	1·0270	144·2	—	—	2/5	7	Climax	—	—
8/7/08	,,	,,	,,	,,	101·2	101·8	33½	—	1·0503	1·0237	147·7	—	—	1/3	12	End	Dikkop ?	28
10/7/08	,,	,,	,,	,,	100·6	101·6	37	—	1·0514	1·0212	147·4	1·58	1·34358	2/5	14	,,	Dikkop	38
13/7/08	,,	,,	,,	,,	Norm.	Norm.	37½	3·86	1·0518	1·0217	140·0	1·67	1·34386	2/5	17	Past	Heart failure	36
26/6/08	3704	Geld.	16 yrs.	Fairly good	Norm.	Norm.	32½	3·71	1·0489	1·0253	143·4	1·74	—	1/2	—	Before inj.	—	—
3/7/08	,,	,,	,,	,,	102·0	103·6	30	2·69	1·0469	1·0237	140·9	—	—	2/5	7	Climax	—	26
8/7/08	,,	,,	,,	,,	101·6	103·0	34	—	1·0485	1·0211	144·0	—	—	1/3	12	End	Dikkop	32
10/7/08	,,	,,	,,	,,	99·0	100·2	35½	—	1·0476	1·0193	144·7	1·49	1·34213	2/5	14	,,	,,	34
13/7/08	,,	,,	,,	,,	Norm.	Norm.	25	3·14	1·0426	1·0218	144·3	1·67	1·34374	2/5	17	,,	,,	42
24/6/08	3457	Geld.	10 yrs.	Very good	104·6	106·6	29	3·09	1·0461	1·0234	141·5	1·84	—	2/5	5	Climax	—	—
25/6/08	,,	,,	,,	,,	105·0	106·0	33	3·86	1·0474	1·0234	141·1	1·70	—	1/3*	6	,,	—	44
29/6/08	,,	,,	,,	Good	100·4	101·2	36	3·12	1·0473	1·0190	137·5	—	—	1/10*	10	End	Dikkop	74
2/7/08	,,	,,	,,	Very good	101·0	101·8	27½	2·93	1·0415	1·0201	135·7	—	1·34423	1/3†	13	,,	,, disap.	50
24/6/08	3400	Geld.	10 yrs.	Fairly good	101·6	103·4	29	—	1·0490	1·0270	140·5	—	—	2/5	9	Beginning	—	38
30/6/08	,,	,,	,,	,,	100·0	102·0	38	—	1·0479	1·0189	139·6	—	—	2/5	15	End	Dikkop	70
3/7/08	,,	,,	,,	,,	102·0	103·8	25½	2·70	1·0415	1·0208	136·5	—	—	2/5	18	,,	,,	78
10/7/08	,,	,,	,,	,,	Norm.	Norm.	24	—	1·0460	1·0274	140·3	2·06	1·34784	2/5	25	Past	,, disap.	—
9/6/08	3522	Geld.	8 yrs.	—	103·0	102·2	30	—	1·0448	1·0248	138·3	1·78	—	2/5	11	Climax	—	52
11/6/08	,,	,,	,,	—	99·8	101·0	28	3·28	1·0460	1·0232	146·3	1·69	—	3/5	13	End	Dikkop	46
12/6/08	,,	,,	,,	—	100·0	101·0	29½	3·46	1·0469	1·0237	143·9	1·68	—	2/5	14	,,	,,	44
13/6/08	,,	,,	,,	—	Norm.	Norm.	28	3·48	1·0468	1·0248	143·0	1·86	—	1/2	15	Past	,, disap.	42
16/6/08	,,	,,	,,	—	,,	,,	27½	3·55	1·0472	1·0256	146·2	1·83	—	2/5	18	,,	—	—

* Orange. † Opalescent.

Horse-Sickness—Averages from all Experiments.

	Blood.				Serum.				
	Temperature. F.	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^{-4}	Viscosity at 25° C.	Surface Tension at 37° C.	Index of Refraction at 37° C.
Number of examinations	194	100	85	90	100	100	83	23	15
„ animals	62	72	58	57	62	62	67	23	14
Average	*102·3*	*34*	*3·68*	*1·0494*	*1·0233*	*142·3*	*1·78*	*5·85*	*1·34368*
Average for normal horses	100·5	33·4	3·80	1·0521	1·0261	146·8	1·83	5·95	—
Difference from average for normal horses	+1·8 %	+1·8 %	−3·2 %	−0·26 %	−0·27 %	−3·1 %	−2·7 %	−1·7 %	—
Maximum	106·4	58	7·39	1·0655	1·0300	150·3	2·77	6·17	1·34653
Minimum	97·0	23	2·70	1·0412	1·0189	130·9	1·49	4·98	1·34213
	%	%	%	%	%	%	%	%	%
Variation above average	4·0	70·5	100·5	1·53	0·65	5·6	56	5·5	0·21
„ below „	4·2	32·5	26·5	0·78	0·43	8·0	16	14·9	0·12
„ total	8·2	103	127	2·31	1·08	13·6	72	20·4	0·33
„ above normal average	5·9	74	94	1·27	0·38	2·4	51	3·7	—
„ below „ „	3·5	31	29	1·04	0·70	10·8	19	16·3	—
Values above average	—	46	40	42	56	47	52	65	53
„ below „	—	54	60	58	44	53	48	35	47
„ above normal average	—	49	34	23	10	12	35	48	—
„ below „ „	—	51	66	77	90	87	65	52	—

Specific gravity, conductivity, viscosity, surface tension, of a greater number of sick than of normal horses, range below the respective normal averages. The limits of variations of all serum values of sick horses are wider than normal. The index of optical refraction, compared with that of 3682 and 3685, seems to be subnormal.

More normal values for comparison are yet missing.

An arrangement of the values of sick horses, according to the latitudes of variations, in descending order :—

Viscosity blood, vol. blood corp., viscosity serum, surface tension serum, conductivity, specific gravity blood, specific gravity serum, index of refraction,

gives the same series as normal horses.

Though horse-sickness is not a disease of the erythrocytes it provokes a slight decrease of them, which is specially distinct when the attack has passed and the temperature is normal again. The recovery of the globules follows very slowly, and so it happens that even immune horses, examined weeks or months after the sickness, do not give an average of the volume of blood corpuscles that is equal to the normal.

Besides loss of erythrocytes, general impoverishment of the blood liquid takes place, emphasised by decrease of the specific gravity and viscosity of the serum. This points to a diminution of colloids ; and the falling of the conductivity indicates a decrease of the electrolyte-concentration as well.

Specific gravity, viscosity, and conductivity of serum recover quicker than specific gravity of blood and volume of corpuscles, for the former values have regained normal height in immune horses.

AVERAGE VALUES FROM VARIOUS STAGES OF HORSE-SICKNESS COMPARED WITH THE AVERAGES OF NORMAL AND IMMUNE HORSES.

	Blood.						Serum.					
	Volume of Blood Corpuscles.		Viscosity at 25° C.		Specific Gravity at 37° C.		Specific Gravity at 37° C.		Viscosity at 25° C.		Conductivity at 37° × 10^{-4}	
	*		*		*		*		*		*	
Normal horses	100	33·4	90	3·80	48	1·0521	50	1·0261	81	1·83	50	146·8
Horse-sickness, climax	60	34·3	43	3·95	55	1·0500	60	1·0238	39	1·77	60	141·7
„ end	33	33·3	19	3·48	32	1·0483	33	1·0218	15	1·69	33	143·0
„ past	25	30·0	15	3·35	24	1·0475	25	1·0244	13	1·83	25	144·8
„ immunised and hyperimmunised ..	40	32·1	14	3·80	18	1·0500	20	1·0262	14	1·90	20	146·1

* These columns contain the numbers of examinations.

TABLE SHOWING THE FREQUENCY OF MINIMUM, AVERAGE, AND MAXIMUM VALUES IN NORMAL HORSES AND IN THE VARIOUS STAGES OF HORSE-SICKNESS.

			Normal.	Horse-Sickness.			
				Climax.	End.	Past.	Immune and Hyper-immune.
Volume of Blood Corpuscles.	Below normal minimum	22	0 = 0 %	0 = 0 %	0 = 0 %	1 = 4 %	0 = 0 %
	„ „ average	33·4	52 = 52	33 = 55	16 = 49	20 = 80	12 = 60
	Above „ „	33·4	48 = 48	27 = 32	15 = 45	4 = 16	8 = 40
	„ „ maximum	43	0 = 0	8 = 13	2 = 6	0 = 0	0 = 0
Blood. Specific Gravity at 37° C.	Below normal minimum	1·0447	0 = 0 %	4 = 7 %	7 = 22 %	3 = 12 %	1 = 6 %
	„ „ average	1·0521	26 = 54	36 = 66	20 = 63	19 = 80	12 = 66
	Above „ „	1·0521	22 = 46	13 = 24	4 = 12	2 = 8	5 = 28
	„ „ maximum	1·0605	0 = 0	2 = 3	1 = 3	0 = 0	0 = 0
Blood. Viscosity at 25° C.	Below normal minimum	2·95	0 = 0 %	2 = 4 %	4 = 21 %	1 = 7 %	0 = 0 %
	„ „ average	3·80	37 = 41	23 = 54	10 = 53	11 = 73	6 = 43
	Above „ „	3·80	53 = 59	15 = 35	4 = 21	3 = 20	8 = 57
	„ „ maximum	5·27	0 = 0	3 = 7	1 = 5	0 = 0	0 = 0

				%	%	%	%	%
Serum. Viscosity at 25° C.	Below normal minimum	1·55	0 = 0	2 = 5	2 = 13	0 = 0	0 = 0
	„ „ average	1·83	51 = 63	26 = 67	12 = 80	5 = 39	3 = 21
	Above „ „	1·83	30 = 37	21 = 28	0 = 0	8 = 61	10 = 72
	„ „ maximum	2·13	0 = 0	0 = 0	1 = 7	0 = 0	1 = 7
				%	%	%	%	%
Serum. Specific Gravity at 37° C.	Below normal minimum	1·0226	0 = 0	15 = 25	23 = 70	4 = 16	0 = 0
	„ middle between {minimum / average} ..	1·02435	6 = 12	19 = 31	7 = 21	8 = 32	3 = 15
	„ normal average	1·0261	20 = 40	21 = 35	2 = 6	9 = 36	6 = 30
	Above „ „	1·0261	21 = 42	4 = 7	0 = 0	4 = 16	9 = 45
	„ middle between {average / maximum} ..	1·02835	3 = 6	1 = 2	1 = 3	0 = 0	2 = 10
	„ normal maximum	1·0306	0 = 0	0 = 0	0 = 0	0 = 0	0 = 0
				%	%	%	%	%
Conductivity at $37^\circ \times 10^{-4}$	Below normal minimum	140·5	0 = 0	20 = 33	9 = 27	5 = 20	0 = 0
	„ middle between {minimum / average} ..	143·65	12 = 24	25 = 42	8 = 25	5 = 20	5 = 25
	„ normal average	146·8	19 = 38	10 = 17	10 = 31	3 = 12	8 = 40
	Above „ „	146·8	13 = 26	5 = 8	6 = 18	12 = 48	7 = 35
	„ middle between {average / maximum} ..	153·6	6 = 12	0 = 0	0 = 0	0 = 0	0 = 0
	„ normal maximum	160·4	0 = 0	0 = 0	0 = 0	0 = 0	0 = 0

3.—Immune and Hyperimmune Horses (against Horse-Sickness).

Immunised and Hyperimmunised.

Date.	Number.	Sex.	Age.	Condition.	Blood.			Serum.					Pulse.
					Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° C × 10^{-4}	Viscosity at 25° C.	Surface Tension at 37° C.	Amount of Serum (Blood = 1)	
9/6/08	1162	Gelding	11 yrs.	—	32	—	1·0508	1·0271	145·7	1·90	—	½	26
9/6/08	1288	,,	17 ,,	—	31	—	1·0495	1·0287	143·8	1·98	—	½	28
9/6/08	1085	Mare	Aged	—	39	—	1·0537	1·0264	144·2	1·88	—	½	26
9/6/08	1660	Gelding	,,	—	23	—	1·0444	1·0263	153·2	1·89	—	½	28
9/6/08	1672	,,	,,	—	42	—	1·0574	1·0273	150·0	1·89	—	½	29
13/6/08	1972	,,	,,	—	32½	3·77	1·0506	1·0268	142·5	1·99	5·44	½	24
13/6/08	1293	,,	,,	—	24½	3·29	1·0451	1·0259	149·0	1·85	5·91	½	27
13/6/08	3451	,,	6 yrs.	—	30	3·50	1·0481	1·0241	150·2	1·92	5·83	½	26
22/3/08	2903	—	—	—	35	—	1·0501	1·0273	148·5	—	—	—	40
17/6/08	3081	Gelding	11 yrs.	Very poor	34½	—	1·0497	1·0259	142·0	—	—	½	40
17/6/08	3408	,,	Aged	Very good	34½	—	1·0539	1·0263	143·7	1·95	6·07	½	40
17/6/08	3411	,,	10 yrs.	Very poor	36	—	1·0530	1·0250	145·8	2·18	6·27	½	40
17/6/08	3444	,,	11 ,,	Fairly good	35½	—	1·0503	1·0236	143·3	—	—	⅖	40
17/6/08	3449	,,	Aged	Very poor	30½	3·34	1·0475	1·0231	146·6	1·72	5·94	⅓	50
18/6/08	3465	,,	6 yrs.	Poor	24	—	1·0450	1·0257	145·7	—	—	½	40
18/6/08	3493	,,	14 ,,	Fairly good	28½	—	1·0476	1·0258	141·9	—	—	½	40
24/7/08	3340	,,	7 ,,	,,	27½	—	1·0485	1·0268	147·7	—	—	⅖	24
24/7/08	3583	,,	7 ,,	,,	32	—	1·0539	1·0296	142·1	—	—	⅖	24
23/11/07	2915	—	—	—	36	—	—	1·0271	146·5	—	—	—	45
21/11/07	2917	—	—	—	26	—	—	1·0252	149·0	—	—	—	45

Date.	Number.	Sex.	Age.	Condition.	Remarks.	Blood.			Serum.				
						Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° C × 10^{-4}	Viscosity at 25° C.	Surface Tension at 37° C.	Amount of Serum (Blood = 1).
17/6/08	3408	Geld.	Aged	Very good	Injected forty days ago	34½	—	1·0539	1·0263	143·7	1·95	6·07	½
18/6/08	,,	,,	,,	,,	Infused 10,000 c.c. the day before	42	—	1·0568	1·0284	139·4	—	—	½
17/6/08	3411	Geld.	10 yrs.	Very poor	Injected forty days ago	36	—	1·0530	1·0250	145·8	2·18	6·27	½
18/6/08	,,	,,	,,	,,	Infused 10,000 c.c. the day before	37	—	1·0538	1·0258	137·9	—	—	½
26/6/08	3076	Geld.	17 yrs.	Rather poor	Hyperimmunised five months ago; nine times bled since; bled last time four weeks ago	29	3·30	1·0471	1·0248	147·8	1·84	—	⅓
,,	,,	,,	,,	,,	Infused 5,000 c.c.; bled half an hour afterwards	29½	3·43	1·0468	1·0251	143·0	1·85	—	⅖
27/6/08	,,	,,	,,	,,	Infused 10,000 c.c. the day before	34	3·79	1·0524	1·0268	143·9	2·04	—	½
2/7/08	,,	,,	,,	,,	,, ,, six days ago	29	3·56	1·0492	1·0262	141·6	2·01	5·68	⅖
26/6/08	3079	Geld.	12 yrs.	Fairly good	Hyperimmunised five months ago; nine times bled since; bled last time four weeks ago	28	3·02	1·0464	1·0244	148·9	1·80	5·82	½
,,	,,	,,	,,	,,	Infused 5,000 c.c.; bled half an hour afterwards	30	2·97	1·0454	1·0251	142·7	1·77	5·19	½
27/6/08	,,	,,	,,	,,	Infused 10,000 c.c. the day before	32½	2·99	1·0487	1·0262	142·3	—	—	⅖
2/7/08	,,	,,	,,	,,	,, ,, six days ago	31½	3·09	1·0478	1·0246	143·6	—	—	⅖
27/6/08	3119	Geld.	12 yrs.	Rather poor	Infused 5,000 c.c. the day before	31½	3·74	1·0505	1·0269	148·8	1·97	5·78	½
,,	,,	,,	,,	,,	,, 5,000 c.c.; bled half an hour afterwards	27	3·18	1·0483	1·0262	149·7	1·82	5·81	½
28/6/08	,,	,,	,,	,,	,, 10,000 c.c. on June 26 and 27 ..	28	—	1·0470	1·0254	139·0	1·91	—	⅖
2/7/08	,,	,,	,,	,,	,, 10,000 c.c. ,, ,, ..	24	3·04	1·0444	1·0252	142·9	1·90	5·85	⅖
27/6/08	3146	Geld.	11 yrs.	Poor	Infused 5,000 c.c. the day before	36½	3·78	1·0532	1·0263	143·7	1·93	5·57	½
,,	,,	,,	,,	,,	,, 5,000 c.c.; bled half an hour afterwards	34½	3·00	1·0524	1·0262	143·5	1·86	5·75	½
28/6/08	,,	,,	,,	,,	,, 10,000 c.c. on June 26 and 27 ..	40	—	1·0561	1·0275	146·2	2·01	—	½
2/7/08	,,	,,	,,	,,	,, 10,000 c.c. ,, ,, ..	31	3·28	1·0490	1·0255	139·7	1·80	5·44	⅖
24/7/08	3340	Geld.	7 yrs.	Fairly good	Injected twenty-four days ago	27½	—	1·0485	1·0268	147·7	—	—	⅖
26/7/08	,,	,,	,,	,,	Infused 10,000 c.c. on July 24 and 25 ..	31	—	1·0515	1·0283	147·2	—	—	⅖

Immune and Hyperimmunised Horses—Averages from all Experiments.

	Blood.			Serum.			
	Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37°×10⁻⁴	Viscosity at 25° C.	Surface Tension at 37° C.
Number of examinations	40	14	18	20	20	14	10
„ animals	37	12	18	20	20	14	10
Average	*32·1*	*3·80*	*1·0500*	*1·0262*	*146·1*	*1·90*	*5·89*
„ for normal horses	33·4	3·80	1·0521	1·0261	146·8	1·83	5·95
Difference from average for normal horses	−3·9 %	0	−0·20 %	−0·01 %	−0·5 %	+3·8 %	−1·0 %
Maximum	42	4·70	1·0574	1·0296	153·2	2·18	6·27
Minimum	23	3·05	1·0444	1·0231	141·9	1·72	5·44
	%	%	%	%	%	%	%
Variation above average	31	32·5	0·70	0·33	4·8	14·5	6·5
„ below „	28	11·0	0·53	0·30	2·9	9·5	7·6
„ total	59	43·5	1·23	0·63	7·7	24	14·1
„ above normal average	26	32·5	0·50	0·34	4·4	19	5·4
„ below „ „	31	11	0·73	0·29	3·3	6	8·6
Values above average	48	57	50	55	45	43	50
„ below „	52	43	50	45	55	57	50
„ above normal average	45	57	28	55	35	36	20
„ below „ „	55	43	72	45	65	64	80

The physical-chemical peculiarities of blood and serum of animals which possess immunity against horse-sickness differ but very little from normal. Volume of blood corpuscles and specific gravity of blood are only slightly subnormal, while the average viscosity even is somewhat above the normal value.

Hyperimmunisation consists in infusing great quantities of blood from a highly sick into an immune horse. The following are the physical-chemical alterations of immune blood and serum which have been mixed with pathological blood in vivo (examination made shortly before and after hyperimmunisation) :—

In all instances the volume of blood corpuscles is increased after infusion. The differences of the values before and after hyperimmunisation are, however, not greater than normal variations—but still I believe that the increase has to be ascribed to the infusion, as it is met in each case, and it is quite natural that it is so. After an absolute increase of the blood quantity, a certain amount of blood liquid is diffusing out of the vessels in order to prevent a fatal increase of the blood pressure. Therefore the blood becomes more concentrated with regard to blood corpuscles.* A few days after the operation the volume of globules has gone down again.

The same is to be said about the specific gravity of blood and serum. It increases after the first or second hyperimmunisation (except 3119), but also these increases could range within normal limits.

In all cases, except one, the conductivity of serum is lower after the second infusion than it was before the first one. The simultaneous increase of specific gravity explains the phenomenon. The abovementioned diffusion will chiefly refer to water and christalloids. Colloids, however, which diffuse not so easily, will be kept back and increase the specific gravity and at the same time the resistance for the migration of ions. For the same reason the viscosity should increase what apparently proves to be right.

The descending order of variations is the following :—

Vol. blood corp., viscosity blood, viscosity serum, surface tension serum, conductivity, specific gravity blood, specific grarity serum,

that is to say, the same as in normal horses.

(4) Serum Horses.

From these horses which had been hyperimmunised, great quantities of blood were taken in almost regular intervals, and therefore they are from a physical-chemical standpoint horses with artificial anaemia, and the results are of general pathological interest.

* Compare *Tigerstedt*, Ergebnisse der Physiologie, 1907.

Date.	Number.	Sex.	Age.	Condition.	Remarks.	Blood.			Serum.					
						Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^4	Viscosity at 25° C.	Surface Tension at 37° C.	Index of Refraction at 37° C.	Amount of Serum (Blood = 1).
9/6/08	1162	Geld.	11 yrs.	—	Hyperimmunised 26 days ago ..	32	—	1·0508	1·0271	145·7	1·90	—	—	1/2
17/6/08	,,	,,	,,	Poor	Bled on June 9, 6 litres	25½	2·9	1·0440	1·0260	147·1	1·76	5·94	—	1/2
29/6/08	,,	,,	,,	Rather poor	,, ,, 18, 4 ,, ...	24	2·45	1·0424	1·0247	148·7	1·59	—	1·34481	1/2
1/7/08	,,	,,	,,	Poor	,, ,, 30, 6 ,, ...	20	2·23	1·0377	1·0244	149·7	—	—	—	1/3
13/7/08	,,	,,	,,	Rather poor	—	24	2·51	1·0405	1·0227	146·1	1·59	—	1·34395	2/3
14/7/08	,,	,,	,,	,,	Bled on July 13, 4 litres	21	2·43	1·0391	1·0223	156·8	1·55	—	1·34390	2/3
9/6/08	1288	Geld.	17 yrs.	—	Hyperimmunised 28 days ago ..	31	—	1·0495	1·0287	149·7	1·98	—	—	1/2
17/6/08	,,	,,	,,	Fairly good	Bled on June 9, 6 litres	23½	2·9	1·0430	1·0257	150·0	1·82	5·93	—	1/2
29/6/08	,,	,,	,,	,,	,, ,, 18, 4½ ,, ...	23	2·72	1·0421	1·0257	148·0	1·79	5·83	—	1/2
1/7/08	,,	,,	,,	,,	,, ,, 30, 6 ,, ...	19½	2·25	1·0378	1·0227	150·8	1·58	5·92	1·34324	2/5
13/7/08	,,	,,	,,	,,	—	22	—	1·0437	1·0251	151·0	1·68	—	1·34541	2/3
14/7/08	,,	,,	,,	,,	Bled on July 13, 6 litres	20	2·42	1·0402	1·0236	158·3	1·58	—	1·34453	2/3
9/6/08	1085	Mare	Aged	—	Hyperimmunised 26 days ago ..	39	—	1·0537	1·0264	144·2	1·88	—	—	1/2
17/6/08	,,	,,	,,	Good	Bled on June 9, 6 litres	28½	3·21	1·0445	1·0238	142·1	1·79	5·99	—	1/2
29/6/08	,,	,,	,,	Fairly good	,, ,, 18, 4 ,, ...	24	2·66	1·0428	1·0243	150·5	1·70	—	—	1/2
1/7/08	,,	,,	,,	,,	,, ,, 30, 5 ,, ...	20½	—	1·0372	1·0217	150·0	—	—	—	1/2
13/7/08	,,	,,	,,	,,	—	26	—	1·0430	1·0234	150·0	1·60	—	1·34463	2/3
14/7/08	,,	,,	,,	,,	Bled on July 13, 4 litres	22½	2·58	1·0392	1·0221	156·8	1·57	—	1·34388	2/3
9/6/08	1660	Geld.	Aged	—	Hyperimmunised 28 days ago ..	23	—	1·0444	1·0263	147·2	1·89	—	—	1/2
17/6/08	,,	,,	,,	Fairly good	Bled on June 9, 6 litres	18½	2·37	1·0367	1·0219	145·4	1·59	6·28	—	1/2
29/6/08	,,	,,	,,	Poor	,, ,, 18, 6 ,, ...	18½	2·61	1·0378	1·0230	145·8	1·80	6·08	—	1/3
1/7/08	,,	,,	..	,,	,, ,, 30, 4 ,, ...	17	1·97	1·0353	1·0219	147·0	1·48	6·03	1·34283	2/5
13/7/08	,,	,,	..	Rather poor	—	17½	2·30	1·0347	1·0208	145·9	1·58	—	1·34277	1/2
14/7/08	,,	,,	..	Poor	Bled on July 13, 2 litres	17½	2·24	1·0356	1·0215	154·6	1·60	—	1·34279	2/5

9/6/08	1672	Geld.	Aged	—	Hyperimmunised 29 days ago ..	42	—	1·0574	1·0273	150·0	1·89	—	—	1/2
17/6/08	,,	,,	,,	Fairly good	Bled on June 9, 6 litres	32	—	1·0471	1·0236	154·0	—	—	—	1/2
29/6/08	,,	,,	,,	,,	,, ,, 18, 2 ,, ...	27½	3·05	1·0436	1·0229	149·6	1·64	6·02	1·34365	2/6
1/7/08	,,	,,	,,	,,	,, ,, 30, 6 ,, ...	24	2·34	1·0389	1·0208	150·3	1·40	6·04	1·34267	1/2
13/7/08	,,	,,	,,	,,	—	28	—	1·0437	1·0217	153·0	—	—	—	1/2
14/7/08	,,	,,	,,	—	Bled on July 13, 4 litres	24	2·42	1·0394	1·0201	158·6	1·44	—	1·34234	1/2
18/6/08	1293	Geld.	Aged	Fairly good	Hyperimmunised 27 days ago ..	27½	3·39	1·0464	1·0268	145·6	1·96	5·79	—	1/2
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	20½	2·78	1·0407	1·0236	148·8	1·71	5·89	—	1/2
30/6/08	,,	,,	,,	,,	Examined before bleeding	20	2·54	1·0403	1·0250	145·2	—	6·14	1·34723	1/2
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	16	2·05	1·0348	1·0218	146·0	—	—	—	1/2
13/7/08	,,	,,	,,	,,	Examined before bleeding	19	2·34	1·0373	1·0229	144·4	1·66	—	1·34382	2/3
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	16	2·10	1·0337	1·0212	146·4	1·54	—	1·34267	2/3
18/6/08	1972	Geld.	Aged	Fairly good	Hyperimmunised 24 days ago ..	31½	4·22	1·0519	1·0267	146·0	2·04	5·79	—	2/6
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	27	3·26	1·0464	1·0236	149·7	1·75	5·75	—	1/2
30/6/08	,,	,,	,,	,,	Examined before bleeding	26½	3·05	1·0463	1·0250	143·0	—	6·13	—	1/3
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	25	2·61	1·0438	1·0227	145·0	—	—	—	1/2
13/7/08	,,	,,	,,	,,	Examined before bleeding	27	3·17	1·0455	1·0245	146·7	1·72	—	1·34529	2/3
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	26	2·81	—	1·0226	150·0	1·60	—	1·34384	1/2

Date.	Number.	Sex.	Age.	Condition.	Remarks.	Blood.			Serum.					
						Volume of Blood Corpuscles.	Viscosity at 25° C.	Specific Gravity at 37° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10^4	Viscosity at 25° C.	Surface Tension at 37° C.	Index of Refraction at 37° C.	Amount of Serum (Blood = 1).
18/6/08	3451	Geld.	6 yrs.	Fairly good	Hyperimmunised 26 days ago ..	33	3·67	1·0489	1·0239	147·4	1·73	5·65	—	1/2
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	28	3·19	1·0452	1·0214	150·3	1·56	—	—	1/2
30/6/08	,,	,,	,,	,,	Examined before bleeding	29	3·06	1·0459	1·0227	147·8	—	—	—	1/2
,,	,,	,,	,,	,,	6,000 c.c. taken. Examined 2 hours after bleeding	26	2·52	1·0412	1·0203	149·2	—	—	—	1/2
13/7/08	,,	,,	,,	,,	Examined before bleeding	28½	2·97	1·0444	1·0213	150·0	1·55	—	1·34323	2/3
,,	,,	,,	,,	,,	6.000 c.c. taken. Examined 2 honrs after bleeding	30	3·00	1·0437	1·0200	152·5	1·46	—	1·34224	2/3
20/6/08	2270	Geld.	Aged	Good	Bled in past 80 days, 48,000 c.c. Bled 6,000 c.c. 2 days ago	16½	2·26	1·0352	1·0214	158·6	1·49	5·79	—	2/3
20/6/08	2903	,,	9 yrs.	Fairly good	Bled in past 80 days, 48,000 c.c. Bled 6,000 c.c. 2 days ago	23½	2·64	1·0416	1·0224	152·6	1·62	5·75	—	2/3
20/6/08	3091	,,	11 ,,	Good	Bled in past 80 days, 46,000 c.c. Bled 4,000 c.c. 2 days ago	17½	1·97	1·0355	1·0204	155·8	1·47	5·77	—	2/3
20/6/08	3172	,,	13 ,,	Exceedingly poor	Bled in past 80 days, 44,000 c.c. Bled 2,000 c.c. 2 days ago	22	2·75	1·0396	1·0217	150·0	1·59	6·03	—	2/3
22/6/08	3033	,,	15 ,,	Rather poor	B'ed in past 82 days, 44,000 c.c. Bled 6,000 c.c. 4 days ago	17	2·55	1·0352	1·0214	148·7	1·59	5·67	—	2/3
22/6/08	3064	,,	13 ,,	Fairly good	Bled in past 82 days, 48,000 c.c. Bled 6,000 c.c. 4 days ago	20	2·24	1·0370	1·0208	151·2	1·43	5·46	—	1/2
22/6/08	3084	,,	11 ,,	Good	Bled in past 82 days, 48,000 c.c. Bled 6,000 c.c. 4 days ago	25	2·66	1·0375	1·0197	147·5	1·38	5·23	—	1/2

The alterations in the peculiarities of blood and serum caused by loss of blood are considerable.

After the operation also a diffusion of water with salts takes place through the walls of the vessels, like after infusion, but in the reverse direction, namely, from the tissues into the blood.

The consequence is a dilution of the latter, specially with regard to corpuseles and colloids.

The visible results are: Decrease of volume of erythrocytes, of viscosity, and specific gravity of blood and serum, and also of the index of refraction of the latter.

The conductivity increases because the quantity of colloids in the serum is less; they are much slower restituted than electrolytes with their comparative easy diffusibility. The behaviour of the surface tension is not yet sure; sometimes it decreases, sometimes it increases, the latter being probably the rule.

The alterations of blood and of serum caused by the loss of blood, amounting from 12 *to* 16 *litres within twelve or twenty days respectively, are in no instance restituted* ad integrum *in two weeks' time.*

The results obtained from normal, sick, and immune animals by the various methods are collected in the following tables:—

Table showing volume of blood corpuscles, see page 128.

„ „ viscosity of blood and serum, see page 129.

Specific Gravity of Blood and Serum (at 37° C).—Averages from all Values.

	Normal.		Horse-Sickness.		Immune and Hyperimmune.	
	Blood.	Serum.	Blood.	Serum.	Blood.	Serum.
Number of examinations	48	50	90	100	18	20
„ animals	48	50	57	62	18	20
Average	*1·0521*	*1·0261*	*1·0494*	*1·0233*	*1·0500*	*1·0262*
„ for normal horses	1·0521	1·0261	1·0521	1·0261	1·0521	1·0261
Difference from average for normal horses	0	0	−0·26 %	−0·27 %	−0·20 %	+0·01 %
Maximum	1·0605	1·0306	1·0655	1·0300	1·0574	1·0296
Minimum	1·0447	1·0226	1·0412	1·0189	1·0444	1·0231
	%	%	%	%	%	%
Variation above average	0·80	0·44	1·53	0·65	0·70	0·33
„ below „	0·70	0·34	0·78	0·43	0·53	0·30
„ total	1·50	0·78	2·31	1·08	1·23	0·63
„ above normal average	0·80	0·44	1·27	0·38	0·50	0·34
„ below „ „	0·70	0·34	1·04	0·70	0·73	0·29
Values above average	46	48	42	56	50	55
„ below „	54	52	58	44	50	45
„ above normal average	46	48	23	10	28	55
„ below „ „	54	52	77	90	72	45

Conductivity of Serum (at 37° C.) × 10-4.—Averages from all Values.

	Normal.	Horse-Sickness.	Immune and Hyper-immune.
Number of examinations	50	100	20
„ animals	50	62	20
Average	*146·8*	*142·3*	*146·1*
„ for normal horses	146·8	146·8	146·8
Difference from average for normal horses ..	0	−3·1 %	−0·5 %
Maximum	160·4	150·3	153·2
Minimum	140·5	130·9	141·9
	%	%	%
Variation above average..	9·2	5·6	4·8
„ below „	4·3	8·0	2·9
„ total	13·5	13·6	7·7
„ above normal average	9·2	2·4	4·4
„ below „ „	4·3	10·8	3·3
Values above average	40	47	45
„ below „	60	53	55
„ above normal average	40	13	35
„ below „ „	60	87	65

Surface Tension of Serum (at 37° C.).—Averages from all Values.

	Normal.	Horse-Sickness.	Immune and Hyper-immune.
Number of examinations	42	23	10
„ animals	36	23	10
Average	*5·95*	*5·85*	*5·89*
„ for normal horses	5·95	5·95	5·95
Difference from average for normal horses ..	0	−1·7 %	−1·0 %
Maximum	6·45	6·17	6·27
Minimum	5·37	4·98	5·44
	%	%	%
Variation above average..	8·4	5·5	6·5
„ below „	9·8	14·9	7·6
„ total	18·2	20·4	14·1
„ above normal average	8·4	3·7	5·4
„ below „ „	9·8	16·3	8·6
Values above average	57	65	50
„ below „	43	35	50
„ above normal average	57	48	20
„ below „ „	43	52	80

Comparison of the Coefficient of Optical Refraction with other Values.

Horse.	Date.			Index of Refraction at 37° C.	Viscosity at 25° C.	Specific Gravity at 37° C.	Conductivity at 37° × 10-4
3704	10/7/08	Horse-sickness	End	1·34213	1·49	1·0193	144·7
3631	29/6/08	,,	,,	1·34226	1·56	1·0199	141·7
3662	10/7/08	,,	Climax	1·34264	—	1·0191	135·1
3663	10/7/08	,,	End	1·34301	—	1·0191	139·5
3706	10/7/08	,,	,,	1·34308	1·62	1·0203	145·7
3663	13/7/08	,,	Past	1·34311	1·60	1·0218	148·0
3667	10/7/08	,,	End	1·34324	—	1·0193	138·1
3702	10/7/08	,,	,,	1·34358	1·58	1·0212	147·4
3705	10/7/08	,,	,,	1·34366	1·63	1·0198	140·8
3706	14/7/08	,,	,,	1·34372	1·68	1·0217	147·7
3704	13/7/08	,,	,,	1·34374	1·67	1·0218	144·3
3668	10/7/08	,,	,,	1·34395	—	1·0209	138·5
3338	29/6/08	,,	,,	1·34419	1·70	1·0228	142·1
3457	2/7/08	,,	,,	1·34423	—	1·0201	135·7
3475	2/7/08	,,	Past	1·34456	—	1·0242	147·8
3668	13/7/08	,,	,,	1·34473	1·69	1·0232	140·7
3627	2/7/08	,,	,,	1·34473	—	1·0243	149·0
3450	29/6/08	,,	,,	1·34481	1·96	1·0245	146·2
3685	Average for 6 values	Normal	—	1·34502	1·62	1·0238	149·2
3707	10/7/08	Horse-sickness	End	1·34529	1·79	1·0239	141·3
3682	Average for 6 values	Normal	—	1·34549	1·69	1·0247	148·9
3634	8/7/08	Horse-sickness	Past	1·34576	—	1·0243	150·9
3465	8/7/08	,,	,,	1·34642	—	1·0259	146·0
3340	8/7/08	,,	End	1·34653	—	1·0257	141·8
3701	10/7/08	,,	Past	1·34689	1·85	1·0259	147·0
3701	13/7/08	,,	,,	1·34743	1·99	1·0274	138·4
3400	10/7/08	,,	,,	1·34784	2·06	1·0274	140·3

The differences in the physical-chemical properties of blood and serum between normal horses, horses in different stages of horse-sickness, and immune horses are shown by the tables on pages 177, 178, and 179.

Results.

Horses suffering from horse-sickness are distinguished by the following peculiarities from normal horses (average values):—

Volume of blood corpuscles and viscosity of blood are supernormal during the climax, but considerably below normality during the end of the disease and a certain time after it. Specific gravity, viscosity, and conductivity of serum are lower than normal at the climax and at the end of the attack.

The differences between normal and immune horses are as follows:—

The average volume of blood corpuscles is lower than normal. The specific gravity is evidently subnormal, because the average is lower, and 72 per cent. of the values of immune and hyperimmune horses lay below the normal average. The same is the case with the surface tension of serum; eight of ten values are lower than the normal average.

F.—HORSE-SICKNESS.

From the first two horses (2915 and 2917) the following physical-chemical characteristics were examined :—

(1) Temperature of the body.
(2) Volume of erythrocytes.
(3) Viscosity of blood.
(4) Viscosity of serum.
(5) Specific gravity of serum.
(6) Conductivity of serum.
(7) Coefficient of thermical expansion.
(8) Quantity of daily urine.
(9) Viscosity of urine.
(10) Specific gravity of urine.
(11) Conductivity of urine.
(12) Alkalinity of urine.*
(13) Quantity of drinking water.

Both horses were injected on the 8th October, 1907, subcutaneously with 2 c.c. virus CD 2884 and 300 c.c. polyvalent serum 186. Both have to be considered to be immune to a certain extent, for they had passed a slight experimental horse-sickness attack. The examinations were started seventeen days before infection and—as in piroplasmosis—made daily for two months. After an interval of two and a half months they were taken up again—the 9th February, 1908—and in addition to the above-mentioned characteristics, surface tension and specific gravity of blood, and surface tension and alkalinity of serum were investigated, whilst the coefficient of expansion and amount of drinking water were left out.

After hyperimmunisation, the 25th and 26th February, 1908, amounting to 10,000 c.c. virus 3375 in each horse, T strain, 18th gen. (inadequate to immunisation), both contracted horse-sickness, of which 2917 died on 3rd March, 1908, 2915 recovered, but succumbed a few days later to piroplasmosis and sequels of horse-sickness on 9th March, 1908.

* The alkalinity of serum and urine is indicated by the quantity of neutralisation—water *pro* litre serum and urine respectively.

Date.	Temperature.		Blood.		Serum.				Drink-water.	Temperature, 9 a.m.	Urine.					Clinical Observations.
	Morning.	Evening.	Volume of Blood Corpuscles.	Viscosity at 77° F.	Viscosity at 77° F.	Specific Gravity at 98·6.	Coefficient of Expansion 71·6–98·6.	Conductivity at 98·6 × 10.⁴			Quantity.	Viscosity. at 37°	Alkalinity.	Conductivity at 98·6 × 10.⁴	Specific Gravity at 98·6.	
			%													
Sept. 20	—	—	—	—	—	—	—	146·9	—	—	—	—	—	—	—	—
,, 21	99·4	100·8	46	—	—	—	—	—	3	—	—	—	—	—	—	—
,, 22	99·4	100·4	45	—	—	—	—	143·8	8	—	2·5	—	4·0	320	1·024	—
,, 23	99·6	100·6	46	—	—	—	—	145·5	10	—	2·7	—	3·2	336	1·026	—
,, 24	100·0	100·6	45	—	—	—	—	143·5	10	—	4·4	—	2·9	353	1·023	—
,, 25	99·6	101·0	43	—	—	—	—	142·5	10	—	—	—	7·2	536	1·032	—
,, 26	99·4	101·0	42	—	—	—	—	146·7	17·5	—	3·3	—	5·8	453	1·037	—
,, 27	99·6	101·0	43	—	—	—	—	146·2	18	—	2·3	—	6·5	502	1·039	—
,, 28	100·0	101·0	43	—	—	—	—	—	20	—	—	—	—	—	—	—
,, 29	100·6	100·6	—	—	1·78	—	—	146·8	—	—	1·6	—	9·0	664	1·042	—
,, 30	100·6	100·6	41	—	1·73	—	—	147·5	20	—	2·5	—	3·2	392	1·028	—
Oct. 1	100·0	101·6	40	—	1·68	1·0280	—	145·5	8	—	3·8	—	4·0	427	1·024	—
,, 2	100·4	101·4	39	3·65	1·68	1·0275	—	146·7	10	—	3·4	—	6·5	540	1·036	—
,, 3	100·0	101·4	39	4·60	1·80	1·0280	0·00032	147·1	14	70	4·6	—	4·7	445	1·038	—
,, 4	99·2	101·0	40	4·30	1·80	1·0286	0·00032	144·9	16·5	70	—	—	6·8	495	—	—
,, 5	100·2	102·6	39	3·95	—	1·0271	0·00034	144·5	9	67	0·9	—	8·3	611	1·033	—
,, 6	100·4	101·2	39	—	—	1·0283	0·00033	143·8	—	61	2·5	—	7·2	618	1·038	—
,, 7	99·0	100·8	44	—	1·87	1·0300	0·00034	143·3	11	56	4·0	—	4·3	448	1·026	—
,, 8	98·6	101·0	39	4·00	1·68	1·0282	0·00034	144·3	9	61	3·4	—	5·8	465	1·027	Injected subcutaneously: 300 c.c. polyvalent serum 186. 2 c.c. virus CD 2884.
,, 9	100·4	101·4	38	3·90	1·70	1·0274	0·00034	145·5	6	65	4·0	—	5·0	550	1·029	—
,, 10	99·2	101·4	37	4·20	1·80	1·0280	0·00033	144·9	10	72	3·0	—	6·5	606	1·029	—
,, 11	99·6	101·6	36	3·85	1·77	1·0280	0·00033	144·3	11	74	3·8	—	5·4	530	1·029	—
,, 12	100·2	101·0	—	4·15	1·78	—	—	143·2	7	65	3·0	—	—	596	1·032	—
,, 13	99·6	101·0	38	4·05	1·78	1·0288	0·00032	145·4	15	67	3·8	—	4·3	697	1·029	—
,, 14	99·0	101·4	40	4·15	1·78	1·0286	0·00033	146·3	16	67	5·2	—	4·7	680	1·029	—

,,	15	100·0	100·4	38	4·60	1·74	1·0286	0·00033	149·0	15	68	7·0	1·10	3·6	604	1·023	—		
,,	16	99·2	102·4	40	4·65	1·70	1·0299	0·00034	145·4	7	70	5·6	1·05	4·0	587	1·025	—		
,,	17	101·0	104·0	38	3·95	1·72	1·0282	0·00033	148·8	22	74	5·6	1·15	4·3	726	1·028	Pulse	44.	
,,	18	101·8	104·6	37	4·80	1·70	1·0285	0·00034	145·5	16·5	76	—	1·15	5·4	736	1·028	,,	48.	
,,	19	102·4	104·0	37	4·00	1·60	1·0285	—	143·2	18	72	4·4	1·10	4·7	533	1·025	,,	56.	
,,	20	103·2	103·0	36	3·35	1·60	1·0260	0·00033	144·1	4	59	5·0	1·20	3·6	326	1·021	,,	40.	
,,	21	102·0	103·6	34	3·10	1·68	1·0255	0·00033	144·5	12	65	0·9	1·15	7·2	600	1·028	,,	48.	
,,	22	102·6	103·6	36	3·70	1·55	1·0253	0·00032	145·4	14·5	72	2·8	1·18	6·8	508	1·022	—		
,,	23	100·6	102·0	35	3·65	1·70	1·0245	0·00034	148·6	16	77	2·8	1·22	9·7	648	1·036	Pulse	50.	Slight dikkop.
,,	24	99·6	101·0	34	3·55	1·64	1·0237	—	149·3	11	77	2·6	1·35	6·5	513	1·038	,,	56.	
,,	25	99·6	101·0	34	3·30	1·48	1·0240	0·00032	147·8	4·5	70	5·2	1·05	2·5	385	1·023	,,	56.	
,,	26	99·0	100·8	36	3·75	1·38	1·0260	—	147·5	3·5	63	3·7	1·05	4·3	589	1·032	,,	60.	Dikkop disap.
,,	27	99·8	100·8	37	3·75	1·28	1·0261	0·00032	145·5	4·5	63	3·7	1·18	4·7	616	1·031	,,	48.	
,,	28	100·0	100·8	34	3·40	1·66	1·0262	0·00032	147·9	10	68	4·4	1·20	6·1	569	1·035	,,	48.	
,,	29	99·2	102·2	36	3·60	1·67	1·0269	0·00033	144·9	10	68	3·7	1·20	6·1	566	1·038	—		
,,	30	99·2	100·4	36	3·40	1·77	1·0270	0·00033	146·1	6	63	4·0	1·12	6·1	494	1·044	—		
,,	31	99·0	101·8	36	3·40	1·77	1·0271	0·00033	144·1	4·5	63	4·7	1·10	3·1	436	1·021	—		
Nov.	1	99·0	100·6	38	4·50	1·78	1·0275	0·00032	144·1	3	63	3·0	1·20	5·8	632	1·036	—		
,,	2	99·2	100·4	36	4·10	1·70	1·0276	—	146·8	15	67	4·4	1·15	5·0	—	1·035	—		
,,	3	99·0	100·8	37	4·00	1·70	1·0280	0·00032	145·5	10	68	1·9	1·26	5·8	616	1·039	—		
,,	4	98·4	100·6	36	3·60	1·84	1·0276	0·00032	147·2	15	72	2·5	1·15	5·4	586	1·037	—		
,,	5	100·4	101·6	36	4·10	1·74	1·0274	0·00032	146·1	14·5	72	5·6	1·10	4·7	384	1·020	—		
,,	6	98·6	100·8	38	3·95	1·73	1·0276	0·00034	147·4	18	72	3·9	1·05	3·2	658	1·025	—		
,,	7	99·0	102·6	35	3·70	1·85	1·0276	0·00033	145·5	7·5	70	3·0	1·15	6·8	696	1·034	—		
,,	8	99·4	100·0	36	3·60	1·82	1·0271	—	—	8·5	70	3·1	1·25	—	—	—	—		
,,	9	99·0	101·4	—	3·90	1·80	1·0275	—	146·2	21	—	5·6	1·08	—	—	—	—		
,,	10	99·0	100·6	—	4·00	1·80	1·0271	—	—	8	—	3·6	1·08	4·0	443	1·020	—		
,,	11	99·0	101·6	40	4·50	1·80	1·0286	0·00032	143·9	13·5	72	1·9	1·12	6·5	523	1·030	—		
,,	12	99·2	100·4	39	4·65	1·90	1·0274	0·00032	144·7	9	74	2·1	1·15	6·8	557	1·029	—		
,,	13	98·6	101·0	36	4·05	1·68	1·0271	0·00032	147·2	18	76	—	1·05	5·8	508	—	—		
,,	14	99·6	100·8	36	3·90	1·88	1·0272	0·00033	148·9	11	72	1·5	1·24	6·8	687	1·035	—		
,,	15	99·0	100·6	37	4·05	1·75	1·0272	0·00032	146·9	6	67	3·5	1·20	6·8	629	1·042	—		
,,	16	99·6	100·2	34	3·55	1·78	1·0259	—	148·3	16	70	6·9	1·10	3·2	355	1·016	—		
,,	17	99·6	100·6	36	3·55	1·86	1·0271	0·00032	147·4	7	68	4·5	1·12	2·7	359	1·019	—		
,,	18	99·4	100·8	37	3·55	1·66	1·0272	0·00032	144·3	7·5	67	4·3	1·10	5·2	457	1·028	—		
,,	19	99·2	100·8	38	3·80	1·78	1·0271	0·00032	144·1	4·5	67	3·7	1·20	7·2	600	1·037	—		
,,	20	99·4	100·8	35	4·30	1·70	1·0269	0·00032	146·6	8	72	3·8	1·20	6·8	606	1·036	—		
,,	21	98·8	100·4	37	3·90	1·70	1·0271	0·00032	146·3	12	72	2·0	1·15	7·2	632	1·042	—		
,,	22	98·8	102·0	37	4·10	1·80	1·0270	0·00032	144·8	11·5	72	3·1	1·15	6·8	609	1·039	—		
,,	23	99·0	101·0	36	4·20	1·80	1·0271	0·00032	146·5	14	72	3·2	1·20	8·6	754	1·042	—		

Horse 2915.

Date.	Temperature.		Blood.				Serum.		
	Morning.	Evening.	Volume of Blood Corpuscles.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.
Feb. 9	—	—	48	1·0603	6·00	4·83	1·0282	1·95	5·68
,, 10	—	—	50	1·0616	5·58	5·18	1·0287	1·98	5·77
,, 11	—	—	47	1·0644	6·60	4·88	1·0300	2·10	5·17
,, 12	98·6	101·0	48	1·0633	5·72	5·17	1·0301	2·08	5·22
,, 13	99·0	101·0	47	1·0622	5·95	5·17	1·0297	2·11	5·52
,, 14	99·4	101·6	45	1·0625	5·70	4·09	1·0295	2·06	5·62
,, 15	99·0	99·8	45	1·0626	5·80	3·98	1·0295	2·07	5·59
,, 16	98·6	100·0	46	1·0631	6·45	4·44	1·0295	2·10	5·24
,, 17	98·0	100·6	46	1·0641	6·55	5·23	1·0304	2·10	—
,, 18	100·0	101·0	47	1·0620	—	4·93	1·0293	2·10	5·03
,, 19	99·0	100·8	45	1·0603	5·56	4·79	1·0287	1·90	5·30
,, 20	98·0	101·0	46	1·0610	5·60	5·20	1·0292	2·00	4·74
,, 21	99·0	100·4	47	1·0596	5·60	4·56	1·0282	1·82	4·67
,, 22	99·6	101·2	43	1·0586	5·54	4·88	1·0276	1·79	4·22
,, 23	99·8	100·8	44	1·0599	5·70	3·71	1·0283	1·92	4·33
,, 24	100·2	100·6	44	1·0599	5·20	4·78	1·0284	1·78	4·92
,, 25	100·0	102·0	43	1·0588	4·94	5·13	1·0282	1·98	4·12
,, 26	100·8	101·0	45	1·0612	6·35	5·04	1·0294	2·07	4·33
,, 27	100·0	102·0	46	1·0621	5·95	4·04	1·0297	2·01	3·96
,, 28	102·0	103·6	44	1·0595	5·54	4·96	1·0294	1·95	4·12
,, 29	102·0	105·6	41	1·0577	5·36	4·27	1·0287	2·02	4·52
Mar. 1	104·0	105·8	44	1·0575	5·71	3·71?	1·0284	2·09	4·61
,, 2	104·0	105·0	40	1·0552	4·67	4·77	1·0260	1·94	4·18
,, 3	103·0	103·8	49	1·0595	5·86	4·83	1·0270	1·95	3·83
,, 4	100·8	101·8	52	1·0608	5·76	4·44	1·0238	1·84	4·13
,, 5	99·8	100·6	53	1·0637	6·10	5·16	1·0237	1·89	5·02
,, 6	98·4	100·0	54	1·0627	6·50	5·61	1·0229	1·88	3·48
,, 7	99·2	100·0	50	1·0581	5·37	3·79	1·0221	1·86	—
,, 8	100·4	99·6	42	1·0500	4·29	—	1·0221	1·80	5·31?
,, 9	101·2	103·8	33	1·0466	3·71	—	1·0232	1·90	2·42?

HORSE 2915—(*continued*).

Date.	SERUM—(*continued*).			URINE.				
	Conductivity at 98·6×10.⁴	Alkalinity.	Colour.	Quantity.	Specific Gravity at 98·6.	Sp. Gr. at 98·6. (filtered).	Conductivity at 98·6×10.⁴	Conduct. at 98·6×10.⁴ (filtered).
Feb. 9 ..	142·9	0·11	Light golden yellow	—	—	—	—	—
„ 10 ..	148·0	0·11	Yellow	—	—	—	—	—
„ 11 ..	147·3	0·11	„	—	—	—	—	—
„ 12 ..	145·6	0·11	Yellow—Golden yellow	—	—	—	—	—
„ 13 ..	144·6	0·10	Golden yellow	—	—	—	—	—
„ 14 ..	148·9	0·12	Yellow	—	—	—	—	—
„ 15 ..	146·1	0·11	„	—	—	—	—	—
„ 16 ..	145·9	0·11	Golden yellow	3·0	1·036	1·033	688	618
„ 17 ..	144·4	0·12	Yellow—Golden yellow	3·2	1·039	1·033	605	598
„ 18 ..	147·8	0·12	Yellow	3·1	1·042	1·037	593	590
„ 19 ..	147·5	0·12	„	2·5	1·041	1·034	502	497
„ 20 ..	146·1	0·11	Yellow—Golden yellow	3·0	1·035	1·032	538	526
„ 21 ..	145·9	0·10	Yellow	2·8	1·035	1·032	554	545
„ 22 ..	147·2	0·14	Yellow—Golden yellow	3·2	1·034	1·031	660	642
„ 23 ..	145·1	0·13	Yellow	3·3	1·039	1·037	667	651
„ 24 ..	149·5	0·13	„	5·4	1·029	1·026	637	628
„ 25 ..	147·2	0·13	„	2·1	1·029	1·027	684	678
„ 26 ..	145·4	0·10	Golden yellow	4·8	1·030	1·027	663	658
„ 27 ..	148·3	0·13	Yellow	5·0	1·033	1·031	658	650
„ 28 ..	143·8	0·12	„	3·5	1·033	1·030	560	538
„ 29 ..	141·3	0·11	„	3·3	1·031	1·029	688	682
Mar. 1 ..	140·9	0·11	Yellow—Golden yellow	3·3	1·036	1·034	677	666
„ 2 ..	142·1	0·11	Golden yellow	4·0	1·030	1·029	576	562
„ 3 ..	144·0	0·08	„	3·3	1·034	1·032	472	446
„ 4 ..	142·8	0·10	„	0	—	—	—	—
„ 5 ..	146·1	0·06?	Reddish, clear	1·4	1·035	1·033	344	340
„ 6 ..	147·4	0·05?	„	3·7	1·040	1·037	414	395
„ 7 ..	139·3	0·13	Golden yellow	0·9	1·040	1·036	435	426
„ 8 ..	137·0	?	Reddish golden	4·6	1·034	1·032	461	453
„ 9 ..	129·3	?	Haemalyt. red	3·0	1·028	1·025	482	465

HORSE 2915—(*continued.*)

Date.	URINE—(*continued*).					DRINK-WATER.			URINE.
	Alkalinity.	(Sp. Gr. = 1) Quantity	(Sp. Gr. = 1) Quantity (filtered).	Surface Tension at 98·6.	Viscosity. at 77.	10 a.m.	4 p.m.	Total.	Colour.
Feb. 9 ..	—	—	—	—	—	—	—	—	—
„ 10 ..	—	—	—	—	—	—	—	—	—
„ 11 ..	—	—	—	—	—	—	—	—	—
„ 12 ..	—	—	—	—	—	—	—	—	—
„ 13 ..	—	—	—	—	—	—	—	—	—
„ 14 ..	—	—	—	—	—	—	—	—	—
„ 15 ..	—	—	—	—	—	—	—	—	—
„ 16 ..	6·6	108	99	—	—	6	5·5	11·5	Dark yellow
„ 17 ..	6·2	125	106	—	—	10	0	10	Muddy
„ 18 ..	5·7	130	115	—	1·20	7	2	9	„
„ 19 ..	4·4	102	85	5·46	1·25	9	4	13	„
„ 20 ..	4·4	105	96	6·23	1·25	6·5	3	9·5	„
„ 21 ..	5·4	98	90	5·64	1·23	4·5	4·5	9	„
„ 22 ..	5·5	109	99	6·40	1·18	10	4·5	14·5	Normal
„ 23 ..	6·9	128	122	5·44	1·30	2	14	16	„
„ 24 ..	4·1	156	140	5·47	1·21	12	6	18	„
„ 25 ..	5·7	61	57	6·59	1·17	5	12	17	„
„ 26 ..	4·2	144	130	6·17	1·18	5	4·5	9·5	„
„ 27 ..	5·8	165	155	5·92	1·20	11·5	5	16·5	„
„ 28 ..	6·5	115	105	6·20	1·21	10	3	13	„
„ 29 ..	8·8	102	96	5·27	1·21	2·5	1·5	4	„
Mar. 1 ..	9·9	119	112	5·28	1·25	0	5·5	5·5	„
„ 2 ..	7·8	120	116	5·05	1·21	3	2	5	„
„ 3 ..	7·4	112	106	3·75	1·30	6	3	9	Dark yellow
„ 4 ..	—	—	—	—	—	4·5	3	7·5	—
„ 5 ..	5·2	49	46	4·75	1·35	3	6	9	Dark yellow
„ 6 ..	6·1	148	137	5·22	1·37	11·5	4·5	16	„
„ 7 ..	7·8?	36	32	—	1·40	10·5	4·5	15	Yellow brown
„ 8 ..	?	156	147	4·54	1·40	13	13·5	26·5	Dark greenish brown
„ 9 ..	?	84	75	4·52	1·29	2	1	3	Dark reddish brown

HORSE 2915—(*continued*).

Date.	URINE—(*continued*). Colour (filtered).	CLINICAL AND MICROSCOPICAL EXAMINATIONS.
Feb. 9 ..	—	—
,, 10 ..	—	—
,, 11 ..	—	—
,, 12 ..	—	—
,, 13 ..	—	—
,, 14 ..	—	—
,, 15 ..	—	—
,, 16 ..	Brown	—
,, 17 ..	,,	—
,, 18 ..	,,	—
,, 19 ..	,,	—
,, 20 ..	,,	—
,, 21 ..	,,	—
,, 22 ..	,,	—
,, 23 ..	,,	—
,, 24 ..	,,	—
,, 25 ..	,,	Infused 5,000 c.c. virus of horse 3375.
,, 26 ..	,,	,, 5,000 c.c. ,, ,,
,, 27 ..	,,	—
,, 28 ..	,,	—
,, 29 ..	,,	Pulse 46.
Mar. 1 ..	,,	,, 60.
,, 2 ..	,,	,, 48.
,, 3 ..	Dark brown	,, 56. Serum obtained only by pressing out the plasma.
,, 4 ..	—	,, 48. Dikkop above left eye. Serum obtained only by pressing out the plasma.
,, 5 ..	Reddish brown	,, 48. Dikkop. Yellow gelat. infiltr. on con. membr. and injection. Serum obtained only by pressing out the plasma.
,, 6 ..	Brown	,, 52. Dikkop strong. *Piroplasma equi* rare. Serum obtained only by pressing out the plasma.
,, 7 ..	Dark reddish brown	,, 56. *Piroplasma equi* fairly frequent.
,, 8 ..	Dark greenish brown	,, 64. *Piroplasma equi* frequent. Serum obtained only by pressing out the plasma.
,, 9 ..	Dark reddish brown	,, 68. Conjunctiva yellow. *Piroplasma equi* very frequent. Serum obtained only by pressing out the plasma. Died of piroplasmosis and sequel of horse-sickness.

Horse 2917.

Date.	Temperature.		Blood.		Serum.				Drink-water.	Temperature, 9 a.m.	Urine.					Clinical Observations.
	Morning.	Evening.	Volume of Blood Corpuscles.	Viscosity at 77° F.	Viscosity at 77° F.	Specific Gravity at 98·6.	Coefficient of Expansion (71·6–98·6).	Conductivity at 98·6 × 10⁻⁴			Quantity.	Viscosity at 77.	Alkalinity.	Conductivity at 98·6 × 10⁻⁴	Specific Gravity at 98·6.	
Sept. 20	—	—	—	—	—	1·0262	—	146·8	—	—	—	—	—	—	—	—
,, 21	99·8	100·8	38	—	—	—	—	—	5	—	—	—	—	—	—	—
,, 22	99·6	99·6	36	—	—	1·0270	—	147·5	9·5	—	2·6	—	6·1	563	1·048	—
,, 23	99·2	100·6	36	—	—	1·0270	—	147·0	11	—	1·5	—	5·0	478	1·048	—
,, 24	99·6	101·4	33	—	—	1·0256	—	147·3	13·5	—	1·2	—	7·2	647	1·042	—
,, 25	100·2	100·6	32	—	—	1·0264	—	146·3	5·5	—	1·8	—	6·8	560	1·047	—
,, 26	99·0	101·4	32	—	—	1·0272	—	144·2	6	—	1·2	—	8·3	570	1·049	—
,, 27	99·0	102·2	32	—	—	1·0256	—	148·0	5·5	—	2·7	—	9·0	623	1·051	—
,, 28	99·6	100·6	30	—	—	—	—	—	26·5	—	—	—	—	—	—	—
,, 29	100·0	105·0	—	—	1·68	1·0260	—	149·3	—	—	2·4	—	7·2	707	1·052	—
,, 30	101·0	100·6	30	—	1·73	1·0260	—	150·0	25	—	1·0	—	5·4	628	1·047	—
Oct. 1	99·0	102·2	30	—	1·73	1·0255	—	148·0	10	—	0·6	—	5·0	585	1·038	—
,, 2	99·2	100·8	29	3·20	1·70	1·0255	—	148·2	8	—	—	—	—	—	—	—
,, 3	99·0	101·4	29	3·05	1·70	1·0264	0·00031	150·0	16	70	—	—	—	—	—	—
,, 4	100·8	100·6	29	3·10	1·68	1·0264	0·00032	149·0	7	70	—	—	9·7	738	—	—
,, 5	100·6	101·6	29	2·70	—	1·0258	0·00034	150·5	6·5	67	0·3	—	12·6	781	1·059	—
,, 6	100·2	101·6	29	—	—	1·0256	0·00032	145·1	—	61	—	—	—	—	—	—
,, 7	101·0	100·8	31	—	1·72	1·0268	0·00032	146·4	4·5	56	1·4	—	7·2	590	1·055	—
,, 8	99·0	101·2	30	3·20	1·68	1·0263	0·00032	147·9	6	61	1·7	—	5·4	588	1·046	Injected subcutaneously: 300 c.c. polyvalent serum 186. 2 c.c. virus CD 2884.
,, 9	100·6	101·8	29	3·15	1·84	1·0266	0·00031	144·4	6	65	1·7	—	6·1	583	1·045	—
,, 10	100·0	101·0	29	3·60	1·79	1·0264	0·00032	146·8	7	72	1·8	—	7·6	526	1·045	—
,, 11	99·6	101·2	29	3·15	1·68	1·0268	0·00032	147·0	10·5	74	1·8	—	5·0	681	1·043	—
,, 12	100·0	101·6	—	—	1·68	—	—	146·3	3	65	2·1	—	—	694	1·044	—
,, 13	99·2	101·6	27	2·90	1·73	1·0263	0·00033	149·8	9·5	67	—	—	—	—	—	—

,,	14	100·4	103·4	27	3·35	1·68	1·0267	0·00032	149·8	2	67	4·2	—	4·3	733	1·034	—
,,	15	101·0	103·0	27	3·70	1·70	1·0265	0·00033	149·8	5	68	4·6	1·15	5·0	777	1·035	—
,,	16	103·2	104·4	27	3·35	1·60	1·0266	0·00032	149·8	6	70	3·1	1·15	6·8	846	1·039	Pulse 64.
,,	17	104·0	105·6	26	3·25	1·68	1·0255	0·00032	149·7	11	74	1·4	1·32	8·6	764	1·048	,, 62.
,,	18	104·4	105·4	26	2·90	1·60	1·0244	0·00032	147·5	9	76	2·2	1·25	7·2	611	1·042	,, 58.
,,	19	104·0	105·0	27	3·10	1·42	1·0242	—	145·1	10	72	2·0	1·24	5·4	391	1·035	,, 58.
,,	20	104·0	104·6	26	2·65	1·50	1·0226	0·00032	143·5	6	59	1·0	1·20	6·1	440	1·030	,, 54.
,,	21	102·6	104·6	27	2·75	1·54	1·0220	0·00032	143·8	11·5	65	2·7	1·28	7·2	451	1·032	,, 48. Supra orbital cavity filled.
,,	22	103·4	103·0	26	2·85	1·53	1·0210	0·00032	146·8	11	72	1·4	1·20	6·5	390	1·030	Dikkop.
,,	23	100·8	101·2	26	2·95	1·60	1·0224	—	146·8	16	77	2·6	1·26	6·5	365	1·034	Pulse 54.
,,	24	100·6	101·0	23	2·65	1·70	1·0212	—	144·4	8·5	77	2·5	1·32	3·6	268	1·034	,, 52.
,,	25	99·6	102·0	22	2·40	1·43	1·0214	0·00032	147·5	5·5	70	1·7	1·32	5·0	399	1·034	,, 52.
,,	26	98·4	101·2	20	2·45	1·38	1·0226	—	148·6	2·5	63	2·2	1·10	2·5	535	1·032	,, 56.
,,	27	99·4	101·6	20	2·45	1·38	1·0225	0·00032	148·0	6	63	2·0	1·18	3·6	547	1·030	,, 56.
,,	28	99·0	100·2	20	2·40	1·60	1·0242	0·00033	147·5	0	68	1·7	1·22	4·3	683	1·035	,, 50. Dikkop disap.
,,	29	99·4	101·6	21	2·60	1·70	1·0247	0·00034	151·6	1·5	68	1·8	1·20	6·8	680	1·045	—
,,	30	99·0	100·6	21	2·80	1·62	1·0248	0·00032	151·0	5·5	63	1·7	1·20	5·4	662	1·031	—
,,	31	99·6	101·2	22	2·25	1·60	1·0248	0·00032	150·0	5	63	1·1	1·25	5·8	636	1·045	—
Nov.	1	99·2	101·2	21	2·45	1·64	1·0248	0·00033	149·1	3	63	2·3	1·25	5·8	741	1·043	—
,,	2	98·8	100·4	23	2·60	1·68	1·0249	—	150·5	9	67	2·5	1·18	5·4	—	1·040	—
,,	3	99·0	100·8	24	2·80	1·70	1·0252	0·00033	149·4	6	68	—	—	—	—	—	—
,,	4	99·0	101·6	24	2·65	1·70	1·0249	0·00034	149·3	5·5	72	1·1	1·25	9·7	824	1·051	—
,,	5	99·6	101·0	23	3·30	1·70	1·0250	0·00033	151·0	8	72	1·9	1·15	6·1	694	1·046	—
,,	6	98·0	100·8	23	2·80	1·65	1·0250	0·00033	152·8	16·5	72	3·5	1·12	5·8	815	1·040	—
,,	7	99·6	100·2	23	2·70	1·76	1·0254	0·00033	150·3	8	70	2·7	1·18	7·2	785	1·050	—
,,	8	99·4	101·6	23	2·40	1·70	1·0252	—	—	6·5	70	2·0	1·25	—	—	—	—
,,	9	98·4	101·8	—	3·00	1·77	1·0255	—	151·6	14	—	1·7	1·23	—	—	—	—
,,	10	99·8	101·0	—	3·00	1·75	1·0255	—	—	8·5	—	0·7	1·20	7·9	811	1·040	—
,,	11	99·2	103·4	27	3·30	1·72	1·0256	0·00032	142·3	7·5	72	1·6	1·30	10·4	666	1·054	—
,,	12	99·4	102·2	25	3·10	1·62	1·0256	0·00033	144·4	4	74	1·9	1·40	7·2	707	1·047	—
,,	13	99·4	101·0	25	3·15	1·70	1·0259	0·00032	149·6	12·5	76	—	1·15	7·2	714	—	—
,,	14	100·0	100·6	26	2·90	1·86	1·0254	0·00032	148·0	14·5	72	2·5	1·25	5·4	703	1·037	—
,,	15	99·4	100·6	27	2·80	1·80	1·0258	0·00033	148·4	2	67	2·0	1·20	6·1	754	1·043	—
,,	16	99·0	101·2	27	3·05	1·68	1·0250	—	148·0	6	70	2·4	1·25	6·5	705	1·044	—
,,	17	99·6	101·6	27	3·05	1·68	1·0264	0·00032	148·0	7	68	2·6	1·25	7·2	685	1·050	—
,,	18	99·0	101·2	26	2·85	1·53	1·0245	0·00032	146·8	3	67	2·0	1·25	7·6	700	1·048	—
,,	19	99·6	101·0	26	2·80	1·76	1·0256	0·00033	146·0	1·5	67	2·6	1·28	8·3	720	1·052	—
,,	20	99·0	101·4	25	3·20	1·70	1·0254	0·00032	151·0	9·5	72	3·0	1·20	7·6	705	1·050	—
,,	21	99·4	100·6	26	3·30	1·65	1·0252	0·00032	149·0	8·5	72	—	1·25	9·7	776	1·053	—
,,	22	99·2	100·6	28	3·40	1·75	1·0260	0·00031	148·0	7·5	72	2·0	1·26	8·6	707	1·055	—
,,	23	99·2	101·0	27	3·20	1·76	1·0250	0·00032	151·6	11	72	1·5	1·25	9·7	877	1·053	—

Date.	Temperature.		Blood.				Serum.						Urine.				
	Morning.	Evening.	Volume of Blood Corpuscles.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Conductivity at 98·6 × 10⁻⁴.	Alkalinity.	Colour.	Quantity.	Specific Gravity at 98·6.	Sp. Gr. at 98·6 (filtered).	Conductivity at 98·6 × 10⁻⁴.	Conduct. at 98·6 × 10⁻⁴ (filtered).
Feb. 9	—	—	36	1·0518	3·73	5·75	1·0260	1·75	5·84	143·0	0·15	Light yellow	—	—	—	—	—
,, 10	—	—	36	1·0540	4·13	5·45	1·0269	1·90	5·56	150·8	0·13	,,	—	—	—	—	—
,, 11	—	—	37	1·0556	4·10	6·53	1·0276	1·90	5·67	153·5	0·11	Yellow	—	—	—	—	—
,, 12	98·6	100·2	37	1·0549	4·08	5·27	1·0279	1·90	4·95	150·6	0·15	Light yellow	—	—	—	—	—
,, 13	98·4	100·0	36	1·0544	4·27	5·21	1·0281	1·91	5·55	145·3	0·15	Yellow	—	—	—	—	—
,, 14	99·0	100·8	37	1·0554	4·33	5·67	1·0283	1·94	4·72	149·3	0·15	,,	—	—	—	—	—
,, 15	98·6	100·2	34	1·0547	4·46	5·24	1·0270	1·88	5·65	145·9	0·16	Light yellow	—	—	—	—	—
,, 16	99·0	100·0	36	1·0552	4·36	4·86	1·0273	1·86	5·55	148·0	0·14	Yellow	1·6	1·045	1·043	623	527
,, 17	98·2	100·6	34	1·0527	4·00	3·48	1·0265	1·80	5·83	146·7	0·18	Light yellow	2·4	1·049	1·042	656	632
,, 18	99·0	101·0	36	1·0535	—	4·76	1·0274	1·83	5·54	151·3	0·15	,,	3·3	1·048	1·045	684	677
,, 19	98·8	101·0	35	1·0535	4·27	5·44	1·0274	1·84	4·66	150·4	0·15	Yellow	2·9	1·049	1·044	642	638
,, 20	98·0	101·4	37	1·0525	4·10	4·88	1·0265	1·80	5·28	148·3	0·16	Light yellow	2·4	1·049	1·045	467	467
,, 21	100·0	100·8	37	1·0524	3·80	4·17	1·0264	1·71	4·42	150·5	0·11	,,	2·8	1·048	1·045	503	488
,, 22	100·0	100·6	34	1·0515	4·64	5·38	1·0261	1·71	5·15	149·8	0·14	,,	3·3	1·044	1·042	593	587
,, 23	99·2	101·0	37	1·0524	4·00	5·50	1·0265	1·82	4·18	149·8	0·13	Yellow	2·2	1·045	1·043	582	574
,, 24	99·6	100·6	34	1·0518	3·80	4·68	1·0263	1·79	4·37	153·7	0·13	,,	5·1	1·038	1·035	684	662
,, 25	100·0	100·0	35	1·0522	3·88	5·17	1·0262	1·81	4·22	149·8	0·09	Yellow—Golden yellow	5·4	1·042	1·040	635	612
,, 26	99·8	101·0	37	1·0535	4·23	5·57	1·0261	1·81	3·96	156·1	0·12	Yellow	3·6	1·032	1·031	603	586
,, 27	100·0	103·6	36	1·0532	4·23	4·82	1·0276	1·93	4·72	146·0	0·18	,,	3·2	1·044	1·041	704	680
,, 28	103·6	104·2	37	1·0547	4·33	4·68	1·0281	1·92	3·92	144·4	0·14	,,	3·1	1·046	1·042	634	593
,, 29	104·6	105·6	35	1·0529	4·27	3·46	1·0274	2·02	5·08	139·9	0·13	,,	3·4	1·037	1·035	600	592
Mar. 1	105·2	106·8	37	1·0536	4·48	5·28	1·0277	1·94	5·12	139·1	?	Haemalyt. red, clear	3·8	1·023	1·022	323	305
,, 2	105·0	105·0	35	1·0523	4·70	4·66	1·0265	1·98	5·11	141·1	?	,, ,,	0·2	1·008	1·008	166	156
,, 3	103·6	104·8	45	1·0559	5·64	4·48	—	—	—	—	?	—	0·9	1·015	1·012	321	265

Date.	URINE—(*continued*).					DRINK-WATER.			URINE.		CLINICAL AND MICROSCOPICAL EXAMINATIONS.
	Alkalinity.	(Sp. Gr.—1) × Quantity.	(Sp. Gr.—1) × Quantity (filtered).	Surface Tension at 98·6.	Viscosity at 77.	10 a.m.	4 p.m.	Total.	Colour.	Colour (filtered).	
Feb. 9	—	—	—	—	—	—	—	—	—	—	—
,, 10	—	—	—	—	—	—	—	—	—	—	—
,, 11	—	—	—	—	—	—	—	—	—	—	—
,, 12	—	—	—	—	—	—	—	—	—	—	—
,, 13	—	—	—	—	—	—	—	—	—	—	—
,, 14	—	—	—	—	—	—	—	—	—	—	—
,, 15	—	—	—	—	—	—	—	—	—	—	—
,, 16	·5·5	72	69	—	—	0	12·5	12·5	Dark yellow	Brown	—
,, 17	6·0	118	101	—	—	4·5	0	4·5	Muddy	,,	—
,, 18	4·4	158	148	—	1·25	5·5	4·5	10	,,	,,	—
,, 19	4·1	142	128	6·08	—	5	6·5	11·5	,,	,,	—
,, 20	3·7	118	108	6·00	1·40	2	7	9	,,	,,	—
,, 21	4·2	134	126	6·03	1·33	14	2	16	,,	,,	—
,, 22	4·2	145	139	5·80	1·27	4	8	12	Dark yellow	Dark brown	—
,, 23	4·3	99	95	6·36	1·32	4·5	4·5	9	,,	,,	—
,, 24	4·2	194	178	6·13	1·20	13·5	5	18·5	Normal	Brown	—
,, 25	4·3	227	216	5·66	1·26	6	0	6	Brown	Dark brown	Infused 4,000 c.c. virus of horse 3375.
,, 26	3·9	115	112	6·10	1·22	22	5	27	Dark yellow	,,	,, 6,000 c.c. ,, ,,
,, 27	5·6	141	131	6·13	1·28	0	5	5	Normal	Reddish brown	—
,, 28	5·7	142	130	5·58	1·30	12·5	2·5	15	,,	,,	—
,, 29	7·8	126	119	5·38	1·25	1·5	2·5	4	Dark yellow	Dark brown	Pulse 50.
Mar. 1	?	88	84	4·42	1·27	0	5	5	Very dark greenish brown	Very dark greenish brown	,, 46. Serum obtained only by pressing out the plasma.
,, 2	2·9?	2	2	4·55	1·20	5·5	4·5	10	Greenish brown	Greenish brown	,, 54. Serum obtained only by pressing out the plasma.
,, 3	4·3	14	11	5·00	1·25	11	4·5	15·5	Muddy brown	Dark brown	,, 52. Serum coagulated. Died of horse-sickness

Three other horses, 2903, 2904, and 3091, were infused on the 13th and 14th of February, 1908, each with a total of 10,000 c.c. virus 3332; 2903 showed an indistinct temperature reaction and suffered later from accidental piroplasmosis. 2904 died of horse-sickness on the fourth day after second infusion. Examinations started two days before first infusion were (besides temperature and microscopical appearance of the blood) made on the following blood and serum properties :—

Of 2903 and 2904—

(1) Volume of red blood corpuscles.
(2) Viscosity of the blood and serum.
(3) Surface tension of blood and serum.
(4) Specific gravity of blood and serum.
(5) Conductivity of serum.
(6) Alkalinity of serum.

Of 3091 and 3124, all mentioned under (1), and the values of serum under (2), (3), (4), and (5), and the depression of freezing point. Horse 3124 was infused the 27th February, 1908, with 2750 c.c. virus 3256.

Horse 2903.

Date.	Temperature.		Blood.				Serum.					Colour.	Clinical and Microscopical Examinations.
	Morning.	Evening.	Volume of Blood Corpuscles.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Conductivity at 98·6 × 10^4.	Alkalinity.		
Feb. 11	99·6	101·6	35	1·0538	4·05	5·59	1·0289	1·90	5·61	149·7	0·15	Light yellow	—
„ 12	99·6	101·2	36	1·0522	3·92	4·34	1·0278	1·90	5·60	146·9	0·15	Yellow	—
„ 13	100·0	100·0	35	1·0559	4·41	5·47	1·0289	1·83	4·72	148·3	0·15	„	Infused 3,000 c.c. virus of horse 3332.
„ 14	100·0	102·0	35	1·0544	4·28	5·00	1·0289	1·95	5·21	147·9	0·15	„	Infused 7,000 c.c. virus of horse 3332.
„ 15	99·8	101·0	36	1·0579	5·36	4·12	1·0301	2·00	4·61	145·9	0·15	„	—
„ 16	100·2	102·4	36	1·0555	4·66	4·81	1·0296	1·95	5·25	145·1	0·16	„	—
„ 17	100·0	101·4	36	1·0549	4·55	4·34	1·0296	2·00	4·84	144·9	0·14	„	—
„ 18	101·6	103·0	35	1·0517	—	5·43	1·0276	1·81	5·74	146·9	0·15	Yellow—golden yellow	—
„ 19	100·2	101·0	32	1·0500	3·87	5·09	1·0278	1·83	4·60	143·8	0·10	„ „	—
„ 20	98·6	101·6	31	1·0492	3·80	4·52	1·0285	2·00	3·83	147·7	0·15	Yellow	—
„ 21	99·8	101·6	29	1·0480	3·40	5·28	1·0283	1·90	4·82	146·7	0·12	„	—
„ 22	98·6	101·0	29	1·0487	3·73	3·72	1·0287	1·93	5·02	145·3	0·12	Yellow—golden yellow	—
„ 23	100·2	100·8	31	1·0489	3·20	4·89	1·0280	1·89	4·52	145·4	0·13	Yellow	—
„ 24	99·6	101·0	32	1·0488	3·40	3·76	1·0274	1·82	5·10	146·6	0·09	Golden yellow	—
„ 25	99·0	101·8	32	1·0496	3·73	3·76	1·0279	1·91	4·87	145·8	0·13	Yellow	—
„ 26	98·6	100·8	33	1·0517	3·73	5·58	1·0284	1·96	4·25	148·3	0·12	„	—
„ 27	100·6	101·6	33	1·0498	3·43	4·39	1·0268	1·80	4·88	148·4	0·16	„	—
„ 28	99·8	101·0	32	1·0490	3·43	4·84	1·0268	1·80	5·67	148·5	0·16	„	—
„ 29	100·0	103·4	33	1·0513	3·72	4·58	1·0276	1·99	5·42	145·9	0·20	„	*Piroplasma equi* very rare.
March 1	102·0	103·6	32	1·0498	3·40	3·99	1·0271	1·94	3·90	147·2	0·11	„	—
„ 2	101·2	101·0	33	1·0496	3·74	4·74	1·0276	1·97	2·62	145·5	0·13	Light yellow—yellow	*Piroplasma equi* rare.

Date.	Temperature.		Blood.				Serum.					Colour.	Clinical and Microscopical Examinations.
	Morning.	Evening.	Volume of Blood Corpuscles.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension. at 98·6.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension. at 98·6.	Conductivity at $98·6 \times 10^{-4}$.	Alkalinity.		
March 3	100·2	103·6	35	1·0507	4·08	4·72	1·0285	1·94	4·66	145·8	0·11	Yellow	Pulse 42. *Piroplasma equi* very rare. Serum obtained only by pressing out the plasma.
„ 4	102·6	104·6	34	1·0524	4·13	4·49	1·0287	2·04	5·48	146·5	0·11	Golden yellow	Serum obtained only by pressing out the plasma.
„ 5	100·6	101·8	28	1·0471	3·55	3·72	1·0270	1·88	5·62	143·5	0·10	Yellow	Pulse 64. *Piroplasma equi* very rare.
„ 6	100·4	103·2	28	1·0479	3·69	3·32	1·0275	2·00	5·32	144·5	0·10	„	Pulse 60.
„ 7	101·6	102·4	28	1·0465	3·81	5·01	1·0276	2·03	—	141·6	0·16	„	„ 52. *Piroplasma equi* very rare.
„ 8	99·0	102·0	29	1·0490	3·90	—	1·0284	1·80	5·00	144·8	0·16	„	Pulse 64.
„ 9	99·8	101·4	32	1·0505	4·22	—	1·0287	2·05	3·14?	142·4	0·22	„	„ 46.
„ 10	99·2	101·6	31	1·0501	4·03	—	1·0283	2·20	5·12	145·3	0·20	„	—
„ 11	100·0	100·8	32	1·0517	4·00	—	1·0285	2·10	4·33	145·9	0·20	„	—
„ 12	98·8	101·0	34	1·0537	4·94	2·82	1·0296	2·11	5·14	144·9	0·18	„	—
„ 13	100·0	101·0	33	1·0515	4·13	3·96	1·0285	2·05	4·38	146·7	0·20	„	—
„ 14	98·6	100·8	34	1·0513	4·13	3·49	1·0285	1·88	4·32	146·2	0·20	„	—
„ 15	99·0	100·6	34	1·0504	3·90	3·56	1·0292	1·95	4·78	147·7	0·19	„	—
„ 16	99·0	101·4	34	1·0521	4·22	—	1·0291	2·16	—	149·4	0·19	„	—
„ 17	100·0	100·8	32	1·0498	4·00	—	1·0276	2·10	5·56	147·6	0·19	„	—
„ 18	99·0	101·8	35	1·0520	4·40	5·10	1·0285	2·05	5·70	146·6	0·18	„	—
„ 19	99·0	102·4	33	1·0511	4·30	5·00	1·0281	1·90	4·54	148·8	0·17	„	—
„ 20	99·8	101·0	34	1·0509	3·80	4·54	1·0284	1·90	4·50	150·7	0·17	„	—
„ 21	99·6	100·6	37	1·0525	4·06	3·61	1·0287	1·87	5·51	151·1	0·19	„	—
„ 22	100·2	100·6	35	1·0501	3·77	3·94	1·0273	1·78	2·90	148·5	0·18	„	—

Horse 2904.

Date.	Temperature.		Blood.				Serum.						Clinical and Microscopical Examinations.
	Morning.	Evening.	Volume of Blood Corpuscles.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 9·86.	Conductivity at 98·6 × 10^{-4}	Alkalinity.	Colour.	
Feb. 11	100·0	101·6	37	1·0549	4·20	5·48	1·0272	1·90	5·48	150·2	0·13	Yellow	—
,, 12	99·6	101·0	35	1·0528	4·18	5·94	1·0270	1·82	5·18	148·3	0·12	,,	—
,, 13	100·0	101·0	34	1·0531	4·32	5·01	1·0272	1·81	5·02	149·2	0·13	,,	Infused 3,000 c.c. virus of horse 3332.
,, 14	99·2	106·0	36	1·0554	5·04	5·13	1·0275	1·98	4·92	148·7	0·14	,,	Infused 7,000 c.c. virus of horse 3332.
,, 15	102·0	106·0	38	1·0580	5·11	4·28	1·0288	2·03	4·26	144·2	0·13	,,	—
,, 16	105·0	105·4	33	1·0533	4·56	4·87	1·0267	1·95	5·00	145·7	0·13	,,	Pulse 60. Conjunctiva injected.
,, 17	104·8	105·8	38	1·0568	5·30	4·95	1·0259	1·96	4·83	142·4	0·11	Yellow—Golden yellow	,, 60. Conjunctiva red.
,, 18	105·0	—	65	1·0728	—	4·76	—	—	—	—	—	—	Dikkop. Died of horse-sickness.

Horse 3091.

Date.	Temperature.		Volume of Blood Corpuscles.	Serum.					Clinical and Microscopical Examinations.
	Morning.	Evening.		Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Conductivity at 98·6 × 10-⁸.	Depression of Freezing Point C.	
Feb. 11	99·0	100·6	29	1·0271	1·78	4·75	153·9	0·6370	—
,, 12	98·6	100·8	29	1·0269	1·88	—	148·3	—	—
,, 13	99·2	100·8	28	1·0274	1·85	4·97	149·1	0·6851	Infused 3,000 c.c. virus of horse 3332.
,, 14	99·6	102·6	31	1·0285	1·94	4·74	145·6	0·6185	Infused 7,000 c.c. virus of horse 3332.
,, 15	99·6	100·6	34	1·0289	1·97	5·48	145·4	—	—
,, 16	98·2	101·0	36	1·0292	2·05 ?	5·56	144·2	—	—
,, 17	98·6	100·6	37	1·0309	2·05	5·88	147·6	0·6144	—
,, 18	99·0	100·8	37	1·0293	2·00	5·49	143·8	—	—
,, 19	98·6	100·0	34	1·0281	1·83	4·81	146·2	0·6195	—
,, 20	99·0	100·6	34	1·0282	1·98	4·67	146·6	0·5856	—
,, 21	99·2	100·6	36	1·0281	1·90	5·37	148·0	—	—
,, 22	98·6	101·0	37	1·0287	1·94	4·12	144·8	—	—
,, 23	99·0	100·6	34	1·0283	1·96	5·28	146·7	0·6360	—
,, 24	98·4	102·8	37	1·0281	1·93	3·84	146·2	—	—
,, 25	101·6	104·0	34	1·0279	1·98	3·92	142·9	—	—
,, 26	102·6	104·6	32	1·0276	1·91	5·03	144·8	0·5846	—
,, 27	103·4	104·6	33	1·0273	1·90	5·30	144·7	0·5459	—
,, 28	103·0	104·0	31	1·0279	1·92	4·87	147·4	0·5418	—
,, 29	102·0	104·4	30	1·0269	2·00	3·78	144·3	—	—
March 1	100·6	105·6	34	1·0262	2·15	3·66	142·6	—	—
,, 2	103·0	102·2	28	1·0265	1·91	3·66	142·5	0·6146	—
,, 3	101·6	103·0	29	1·0265	1·85	4·85	145·2	—	*Piroplasma equi* very rare.
,, 4	100·0	101·8	29	1·0284	1·97	4·12	148·9	0·6930	,, ,, ,,
,, 5	98·8	101·2	29	1·0274	1·98	4·43	146·8	—	Pulse 56. *Piroplasma equi* very rare.

„	6	98·4	100·0	27	1·0268	1·98	4·73	146·7	0·5802	*Piroplasma equi* very rare. Serum obtained only by pressing out the plasma.
„	7	98·8	100·6	30	1·0269	1·98	—	146·6	0·6030	Serum obtained only by pressing out the plasma.
„	8	100·0	100·2	30	1·0268	1·97	4·78	148·6	—	Pulse 58. Serum obtained only by pressing out the plasma.
„	9	99·6	101·0	29	1·0269	2·00	4·24 ?	148·9	0·6102	Pulse 56.
„	10	100·0	100·6	28	1·0260	2·00	6·86 ?	150·6	0·6049	—
„	11	100·6	100·0	28	1·0254	1·94	4·71	148·4	0·5674	—
„	12	99·0	101·0	29	1·0268	1·94	4·97	149·9	0·5900	—
„	13	99·0	100·8	29	1·0269	1·90	—	153·1	0·6018	—
„	14	98·6	101·2	28	1·0260	2·03	5·23	149·5	—	—
„	15	99·4	100·2	28	1·0255	1·95	3·99	150·8	—	—
„	16	99·2	101·6	30	1·0266	1·95	—	151·6	0·6098	—
„	17	98·4	100·2	29	1·0255	1·90	4·34	151·8	0·5968	—
„	18	98·6	101·0	31	1·0269	1·98	5·54	151·3	0·6017	—
„	19	98·4	101·4	31	1·0268	1·82	4·96	151·0	0·5781	—
„	20	99·0	100·4	31	1·0266	1·80	5·38	151·8	0·5766	—
	21	99·6	100·0	31	1·0262	1·69	5·56	152·1	0·5915	—
	22	98·6	100·6	30	1·0265	1·71	5·11	150·2	0·5892	—

Horse 3124.

Date.	Temperature.		Volume of Blood Corpuscles.	Serum.					Clinical Observations.
	Morning.	Evening.		Specific Gravity at 98·6.	Viscosity at 77° F.	Surface Tension at 98·6.	Conductivity at 98·6 × 10⁻⁴.	Depression of Freezing Point. C.	
Feb. 11	99·0	101·4	24	1·0292	2·05	5·37	147·3	0·6235	—
,, 12	99·0	100·6	24	1·0293	2·15	5·64	146·6	—	—
,, 13	99·0	100·2	26	1·0295	2·07	4·27	145·4	0·5942	—
,, 14	99·6	101·6	31	1·0284	1·95	4·51	146·6	0·6330	—
,, 15	99·6	101·4	24	1·0290	2·00	5·22	145·3	—	—
,, 16	99·0	101·2	24	1·0287	2·10 ?	4·67	145·5	—	—
,, 17	99·0	101·4	26	1·0300	2·10	5·64	145·4	0·6157	—
,, 18	100·2	100·6	27	1·0295	2·05	5·03	142·4	0·5901	—
,, 19	98·6	101·0	26	1·0284	1·98	5·37	143·5	0·5985	—
,, 20	98·0	101·6	28	1·0301	2·21	4·03	144·5	0·5933	—
,, 21	99·8	101·0	28	1·0288	2·02	2·95	144·6	0·5739	—
,, 22	98·2	101·2	27	1·0288	2·01	4·62	144·2	—	—
,, 23	99·2	101·2	27	1·0283	2·05	4·05	145·1	0·6352	—
,, 24	99·6	101·2	28	1·0288	2·06	5·19	143·6	—	—
,, 25	99·0	101·0	25	1·0288	2·08	5·02	143·4	0·5958	—
,, 26	98·6	100·6	24	1·0288	2·03	5·29	145·4	0·6111	—
,, 27	99·6	104·6	27	1·0289	2·00	4·85	145·6	0·6039	Infused 2,750 c.c. virus of horse 3256.
,, 28	103·8	104·2	27	1·0298	2·10	3·87	140·6	0·5433	—
,, 29	104·2	105·6	29	1·0283	2·01	5·22	143·9	0·5587	—
March 1	102·0	100·6	36	1·0272	1·95	4·67	149·9		Pulse 76. Died of horse-sickness in the night.

Results and Conclusions.

It must be distinguished between

(*a*) horses which are subcutaneously injected with serum and very small quantities of virus (2915 and 2917, first time);
(*b*) horses infused with great quantities of virus (3124, 2904, 2917, second time);
(*c*) infused horses which in addition develop piroplasmosis (2915 second time, 3091, 2903).

Ad (*a*).—*The volume of blood corpuscles* decreases in both horses continually from the beginning of the examinations until the end of the temperature reaction. Then it increases in 2915 slowly without reaching the original height.

In 2917 a pronounced minimum occurs at the end of the temperature reaction, after which the values increase. That means, though horse-sickness is not a disease of the blood corpuscles the latter are affected. These results are in accordance with those mentioned under "Comparative Experiments."

The Viscosity of the Blood behaves similarly to the volume, for reasons already explained in the chapter "Viscosity." The decreases of volume of blood corpuscles and viscosity are, compared with the values in piroplasmosis, very small and occur as a rule at the end of or after the temperature reaction. (See chapter "Comparative Experiments.")

The alterations of the *viscosity of serum* start later than in piroplasmosis and recovery takes place sooner.

The *specific gravity* shows a most distinct reaction in form of a decrease after an "incubation time" of eleven and eight days respectively, and the minimum again coincides with the disappearance of the fever. This dropping of the specific gravity distinguishes itself from that taking place in piroplasmosis by its late appearance and short duration.

The *conductivity of serum* alters very little; it seems to increase first, a slight but distinct decrease emphasises itself about in the highest fever time, that is to say, coincident with the dropping of the specific gravity, not with its minimum. Also, the behaviour of *the urine* during horse-sickness attacks is different from that in piroplasmosis. Polyuria does not appear, adversely: the quantities of urine are very small in both instances.

The pathological alterations of specific gravity, viscosity, conductivity, and alkalinity of urine are very slight. Only in horse 2917 specific gravity and conductivity show distinct decreases, corresponding with the synchronical dropping of specific gravity of serum.

Résumé.—The physical-chemical alterations of blood and serum and urine due to horse-sickness infection consist of a slight decrease of the volume of erythrocytes, of viscosity of blood and serum, of conductivity of serum and (in one case) of urine. The most distinct declination shows the specific gravity of serum. The chronological order of the alterations in horse-sickness is distinct from those in piroplasmosis. In the latter disease the majority of them appear before the fever; in the former, however, the order is the following:—

(1) Temperature reaction.
(2) Alteration of the conductivity.

Synchronical in one instance:

(3) Alterations of specific gravity (and viscosity) of serum.
(4) Alterations of the volume of blood corpuscles and viscosity of blood.

It is, therefore, possible to distinguish by means of physical-chemical methods whether a horse is suffering from piroplasmosis or horse-sickness.

This task would be greatly facilitated for laboratory use by taking a physical-chemical "description" of every horse.

Ad (b).—A doubtless pure case of horse-sickness after infusion is only 3124. In such cases we would have to distinguish

(1) among symptoms merely due to the infusion of a great quantity of homologous blood;

(2) among symptoms caused by the horse-sickness virus.

The symptoms under (2) are so pronounced and the duration of the attack so short that those mentioned under (1) have no chance to emphasise themselves.

The volume of blood corpuscles increases by accumulation of CO_2 in the jugular blood continually until the lethal exitus occurs caused by heart affection. (Heart form of horse-sickness.)

Specific gravity and viscosity of serum decrease on account of a loss of non-electrolytes (and perhaps colloids), as the dropping of osmotic pressure and the increase of conductivity prove.

Ad (c).—These horses show symptoms of horse-sickness and piroplasmosis together, more or less distinctly those of one or the other disease.

The slight decrease of the blood values in 2903, for instance, speaks for horse-sickness, for it follows a temperature reaction. The exacerbations of the depression of freezing point in 3091, however, are more signs of piroplasmosis. The increases of the blood values of 2915, 2917, and 2904 are caused by the horse-sickness virus, the dropping of the same, and of the conductivity of serum in 2915 is due to the influence of piroplasms. The declinations of specific gravity and surface tension of serum in 2915 are like those found in horse-sickness.

Three horses 2904, 2917, and 3124 died, evidently of horse-sickness. The physical-chemical symptoms, *which indicate the fatal end* a short time before death, are in all horses the following:—

(1) Sudden increase of volume of corpuscles, viscosity, and specific gravity of the blood.

(2) Sudden decrease of the specific gravity of serum.

These symptoms of death are just the reverse in horse 2915 which succumbed to piroplasmosis.

(*g*) PIROPLASMOSIS.

The principle of piroplasmosis is destruction of red blood cells, that is to say, haemolysis, caused by endoglobular parasites. The consequence is anaemia or, better, oligocytaemia. Therefore we have to distinguish among the clinical symptoms of a piroplasmatic infection:

Primary symptoms.

(1) Symptoms directly due to the piroplasms (haemolysis, fever).

(2) Symptoms due to the pathologically increased process of haemolysis, i.e. the consequences of blood destruction. (Alterations of osmotic pressure, icterus haemoglobinuria.)

Secondary symptoms.

(3) Symptoms which are merely consequences of the deficiency of erythrocytes. (Poverty, acceleration of pulse, and respiration.)

(4) Symptoms of re-convalescence, reappearance of young red blood cells (poikilocytosis).

As the clinical symptoms are only the consequences of physical and chemical processes within the blood and tissues, we expected alterations in the physical-chemical state of the serum, for instance, of its osmotic pressure and conductivity, as a consequence of the diffusion of electrolytes, and eventually colloids from the dead erythrocytes into the blood liquid and perhaps of secretions and excretions of the piroplasms themselves. Subsequently the urine altered, for the elimination of abnormal blood constituents partially takes place through the kidneys. At least secondary affections of the latter were expected to signalise themselves also by abnormal physical-chemical peculiarities of their excretion.

From the horses 2840, 2841, 2847, and 2848 we obtained the following indications :—

(1) Temperature.

(2) Results of microscopical and clinical observations.

(3) Number and volume of erythrocytes.

(4) Conductivity of serum at 25°.

(5) Depression of freezing point = osmotic pressure of serum.

(6) Conductivity of urine at 25°.

(7) Depression of freezing point = osmotic pressure of urine.

The examinations were made day by day for about five weeks, that is to say, from a few days before infection, continuing throughout the whole attack almost to complete restitution of the animals.

Horse 2840.

Date.	Temperature.		Red Blood Corpuscles.		Serum.		Morning Urine.			Afternoon Urine.			Results of Microscopical Examinations.	Pulse.
	Morning.	Evening.	Number.	Volume.	Conductivity.	Depression of Freezing Point.	Conductivity.	Colour.	Depression of Freezing Point.	Conductivity.	Colour.	Depression of Freezing Point.		
				%										
June 27	—	—	—	—	113·3	0·556	—	—	—	—	—	—	—	—
,, 29	—	—	—	—	116·3	0·558	—	—	—	—	—	—	—	—
July 1	98·2	100·2	—	—	113·8	0·597	—	—	—	—	—	—	—	—
,, 2	100·2	100·2	—	—	113·9	0·544	—	—	—	—	—	—	—	—
,, 3	99·2	100·4	—	—	115·3	0·578	145	—	0·321	—	—	—	Inj. subc. 5 c.c. blood of DE 2564	—
,, 4	100·2	101·0	—	—	109·9	0·998	—	—	—	—	—	—	—	—
,, 5	99·6	100·0	—	—	114·3	0·778	39	—	—	—	—	—	—	—
,, 6	100·2	99·6	—	—	117·0	0·653	208	—	2·437	—	—	—	—	—
,, 7	100·0	100·4	—	—	—	—	—	—	—	—	—	—	—	—
,, 8	99·2	100·6	—	37	116·1	0·551	348	Yellow, clear	2·365	—	—	—	—	—
,, 9	100·2	100·8	7,700,000	36	115·4	0·564	—	—	—	—	—	—	—	—
,, 10	100·2	100·2	—	37	114·2	0·566	259	Clear	2·055	287	Clear	2·001	—	—
,, 11	98·4	101·0	9,000,000	39	110·8	0·550	380	,,	2·410	135	,,	0·795	—	—
,, 12	99·2	101·4	—	34	112·4	0·666	—	—	—	256	Normal	2·341	Mic. exam.: Negative	—
,, 13	100·0	103·4	8,100,000	36	113·1	0·540	—	—	—	—	—	—	,, ,,	—
,, 14	101·0	101·6	—	—	111·8	0·798	242	Brownish, clear	2·668	—	—	0·789	*Piropl. equi*, rare	34
,, 15	99·0	101·0	7,100,000	37	114·0	0·511	?	Reddish	2·755	—	—	—	,, ,, ,,	32
,, 16	99·2	100·4	5,600,000	34	111·7	0·522	—	—	—	265	Normal	2·815	,, ,, ,,	34
,, 17	99·6	100·4	6,900,000	34	115·5	—	270	Yellow, cloudy	3·393	329	,,	—	Mic. exam.: Negative	36
,, 18	99·8	99·0	6,300,000	33	111·7	0·519	—	—	—	290	Clear	2·754	,, ,,	36

,,	19	99·2	100·2	7,300,000	33	111·3	0·512	246	Clear	2·122	—	—	—	,,	,,	32
											114	Normal	—	—	—	—
,,	20	103·0	105·2	7,300,000	33	108·2		222	Normal	1·548	81	Light yellow	0·508	Mic. exam. :	Negative	46
								81	Normal	—	—	—	—	Conjunctiva	yellow	—
,,	21	102·0	101·2	—	—	110·9	0·529	23	Clear	0·875	21	Clear	—	Mic. exam. :	Negative	40
								133	Dark yellow, cloudy	—	—	—	—	—	—	—
,,	22	99·4	100·6	5,900,000	33	109·2	0·521	20	Clear	1·637	64	Dark yellow	—	Mic. exam. :	Negative	30
,,	23	100·0	100·0	—	30	110·5	0·561	297	Normal	2·091	243	Slight cloudy	—	,,	,,	40
,,	24	99·2	99·4	6,400,000	34	116·3	0·567	347	,,	2·302	362	Dark yellow, clear	—	,,	,,	34
								202	Yellow, clear	—	—	—	—	—	—	—
,,	25	98·0	101·0	—	34	111·0	0·525	98	Light yellow, clear	1·089	—	—	—	Mic. exam. :	Negative	—
,,	26	98·6	98·8	6.300,000	32	107·7	0·523	150	Normal	1·111	—	—	—	,,	,,	—
,,	27	97·8	99·2	—	32	111·7	0·580	170	Dark yellow	1·348	96	Clear	—	,,	,,	—
,,	28	98·0	98·8	—	—	114·1	0·572	281	,,	2·123	—	—	—	,,	,,	—
,,	29	98·2	99·2	6,800,000	31	111·8	0·520	—	—	—	—	—	—	,,	,,	—
,,	30	98·8	99·6	—	—	114·2	0·525	—	—	—	306	Normal	2·141	—		—
,,	31	98·6	99·2	7,400,000	34	113·9	0·559	—	—	—	344	Dark yellow	1·973	—		—
Aug.	1	98·8	99·2	—	—	114·1	0·587	252	Dark yellow, clear	1·556	128	Clear	—	—		—
,,	2	98·2	99·0	7,500,000	34	112·9	0·517	—	—	—	250	Normal	1·617	—		—
,,	3	99·0	99·0	—	—	113·8	0·540	—	—	—	337	,,	2·545	—		—
,,	4	—	—	—	—	114·0	0·581	—	—	—	—	—	—	—		—

Discussion.

The incubation period with regard to the *temperature reaction* is nine days. The fever shows two periods, namely: the first, lasting for four days; the second and more pronounced, which starts after an interval of four days, and endures only three days.

Piroplasma equi is observed the eleventh, twelfth, and thirteenth day (after injection), that is to say, at the end of the first fever period and the beginning of the feverless interval.

Number and volume of red blood corpuscles also show two periods of decrease and re-increase. The first decrease starts about the same time when the temperature rises. The first minimum coincides with the beginning of the feverless interval. The days of the most intensive destruction of corpuscles are therefore those where the parasites are present. The second minimum lies at the end of the second fever reaction. Then the number of erythrocytes increases slowly and is thirty days after injection almost normal again.

The reason that the curves of number and volume of blood corpuscles are not going absolutely parallel are, first of all, the technical errors made, especially in counting, then the poikilocytosis; different sizes of erythrocytes, of course, do not affect the result of counting.

The conductivity of serum oscillates between 108 and 117 $\times$ 10^{-4}. The normal value averaged from five examinations before injection amounts to 114·5 $\times$ 10^{-4}. The very minimum of conductivity = 107·7 on the twenty-third day lies, therefore, about 6 per cent. below the normal value, while the total variation of conductivity during the disease amounts to 8·1 per cent.

Two periods of low values of conductivity may be noticed, both coinciding with the fever periods: first from the eighth to the thirteenth day; second from the seventeenth to the twentieth day; then again, a deep precipitation occurs on the twenty-third day, probably corresponding with a third reaction. After that the conductivity "recovers" very quickly and is already normal again the twenty-fifth day.

I want to draw attention to a phenomenon which is almost typical for piroplasmosis, and which never has been observed in horse-sickness; I mean a remarkable decrease of the conductivity of the serum the first or second day after infection, whereafter conductivity returns at once to normal height, at which it remains for the next days of the incubation period.

In horse 2840 this sudden decrease of conductivity seems to be produced by an increase of the non-electrolytes, because the depression of freezing point shows a corresponding rise, and it is well known that non-electrolytes depress the electrolytic dissociation.

The cryoscopy of serum indicates an enormous depression of the freezing point the first day = — 0·998°, corresponding with an osmotic pressure of about twelve atmospheres at 0° or $12\,(1 + \frac{3.7}{273})$ = about fourteen atmospheres at 37°, while the normal depression is — 0·577° according with an osmotic pressure of about eight atmospheres at 37°. The depression returns then to the normal the fifth day. After another increase coinciding with the second part of the first temperature reaction and subsequently with the first period of low conductivity and considerable destruction of blood corpuscles, the depression remains subnormal for eight days, and varies after that from — 0·52° to — 0·58°, almost parallel to increases and decreases of the conductivity. The low value of conductivity and depression of freezing point from the seventeenth to the nineteenth day points to considerable diminution of serum electrolytes.

The conductivity of urine goes, with a few exceptions, parallel with that of serum, that is to say (as the blood for serum was taken in the morning), the more or less electrolytes the serum contains, the more or less of them respectively are eliminated by the kidneys; but it must not be forgotten that dilution of the same amount of electrolytes in a great quantity of urine-water might misrepresent a minus of electrolytes.

Notwithstanding the examinations of urine being started after the infection, I am inclined to consider the first three values of the conductivity as subnormal as a direct consequence of the injection, according to the synchronical changes in serum. The normal conductivity of urine would be about 250×10^{-4}, hence it is much higher than that of serum, and the limits of variations are enormously wide. $(20 - 230 \times 10^{-4}.)$

The concentration of electrolytes in the urine, of course, has to vary on account of their comparatively constant concentration in the serum. In analogy to the appearances of the serum, the conductivity of urine shows periods, the second of which contains the lowest values (seventeenth, eighteenth and nineteenth day), that is just during the second periods of breaking down of blood corpuscles.

The depression of freezing point of urine, going parallel with the conductivity, proves that the osmotically active components of urine are mostly electrolytes. The variations of the osmotic pressure of urine are considerable, namely, according to the variations of depression of freezing point from $-0{\cdot}32°$ to $-3{\cdot}4°$. P = from 4·4 to 46·6 atmospheres at 37°.

Date.	Temperature.		Red Blood Corpuscles.	Serum.			Morning Urine.				Afternoon Urine.				Results of Microscopical Examinations.	Pulse.
	Morning.	Evening.	Number.	Volume.	Conductivity at 25 × 10^6	Depression of Freezing Point.	Conductivity at 25 × 10^6	Colour.	Depression of Freezing Point.		Conductivity at 25 × 10^6	Colour.	Depression of Freezing Point.			
				%												
June 27	—	—	—	—	112·9	0·548	386	—	2·205		—	—	—		—	—
,, 29	—	—	—	—	115·7	0·550	341	—	2·212		—	—	—		—	—
July 1	97·4	100·2	—	—	115·5	0·563	246	—	—		—	—	—		—	—
,, 2	98·0	101·0	—	—	114·2	0·583	245	—	2·194		—	—	—		—	—
,, 3	100·2	100·4	—	—	115·3	0·552	—	—	—		—	—	—		Inj. subc. 5 c.c. blood of DF 2494	—
,, 4	100·0	101·8	—	—	115·0	0·937	—	—	—		—	—	—		—	—
,, 5	100·0	101·4	—	—	111·8	0·520	—	—	—		—	—	—		—	—
,, 6	101·0	99·4	—	—	117·3	0·545	150	—	1·966		—	—	—		—	—
,, 7	99·4	101·2	—	—	—	—	—	—	—		—	—	—		—	—
,, 8	99·8	100·6	9,200,000	40	115·9	0·579	—	—	—		—	—	—		—	—
,, 9	100·6	101·2	—	40	115·8	0·577	—	—	—		—	—	—		—	—
,, 10	99·4	101·2	9,500,000	40	113·2	0·553	—	—	—		—	—	—		—	—
,, 11	100·8	100·2	—	36	113·7	0·780	—	—	—		229	Clear	1·678		—	—
,, 12	100·2	102·6	8,900,000	37	113·4	0·750	296	—	2·579		129	Greenish yellow brown	—		Mic. exam.: Negative	—
							298	Normal	—		—	—	—		—	—
,, 13	101·0	100·2	7,400,000	38	116·3	0·865	300	—	2·349		—	—	—		*Piropl. equi* rare	—
,, 14	100·0	102·2	—	—	113·6	0·757	—	—	—		268	Normal	2·713		,, ,, ,,	—
								Brown	2·789		—	—	—		,, ,, present	44
,, 15	98·8	104·0	8,900,000	36	110·1	0·526	235									
							221	Yellow, cloudy	—		—	—	—		Mucosa orange	—
,, 16	101·0	102·6	6,400,000	30	111·0	{0·505}	136	Red brown, clear	2·647		87	Clear	1·096		*Piropl. equi* present	46
,, 17	103·0	103·6	6,400,000	31	114·5	{0·505}	251	,,	—		224	Red brown, clear	2·715		,, ,, rare	62

,,	18	100·8	100·0	6,500,000	26	113·2	0·518	261	Brown, clear	2·971	143	Brown, clear	2·207	,, ,, ,,		44
,,	19	100·0	102·0	5,000,000	24	113·0		250	Light brown	2·374	—	—	—	,, ,, ,,		44
								287	Red brown	—	—	—	—	—		—
,,	20	100·0	100·2	5,900,000	26	110·9	0·548	185	Normal	—	103	Red brown	0·937	,, ,, ,,		40
,,	21	100·0	100·0	—	—	116·2	0·578	297	Red brown	2·387	40	Light yellow, clear	—	Conjunctiva pale		—
								47	Yellow, clear	—	—	—	—	—		—
								27	,,	—	—	—	—	—		—
,,	22	100·6	103·4	5,600,000	23	112·5	0·525	275	Dark yellow	2·136	151	Dark yellow	—	Mic. exam.: Negative		48
,,	23	100·8	100·4	6,700,000	29	113·3	0·552	290	,,	2·623	180	Dark yellow, clear	—	,, ,,		44
,,	24	100·0	101·0	—	28	118·9	0·603	354	,,	2·449	430	Normal	—	*Piropl. equi* rare		44
								318	Normal	—	—	—	—	—		—
,,	25	100·2	102·0	4,800,000	21	111·6	0·531	130	Dark yellow, clear	2·192	181	Clear	—	,, ,, very rare		—
,,	26	100·0	101·0	—	20	111·5	0·511	210	,,	2·321	134	Normal	—	Mic. exam.: Negative		—
,,	27	99·8	99·6	5,200,000	23	113·5	0·536	258	,,	2·254	143	,,	—	,, ,,		—
,,	28	99·2	101·2	—	—	116·4	0·569	301	—	2·158	—	—	—	—		—
,,	29	99·2	101·0	—	28	113·4	0·676	—	—	—	—	—	—	Mic. exam.: Negative		—
,,	30	99·6	101·0	7,500,000	29	117·8	0·542	375	Normal	2·512	419	Normal, thick	—	—		—
,,	31	100·0	100·2	—	—	113·0	0·928	—	—	—	310	Dark yellow	2·382	—		—
Aug.	1	100·2	100·4	5,400,000	27	116·0	0·537	—	—	—	300	,,	2·408	—		—
,,	2	100·2	100·4	—	31	114·7	0·747	203	Normal	1·286	178	Normal	—	—		—
,,	3	100·0	100·4	6,000,000	28	114·8	0·541	297	,,	2·536	—	—	—	—		—
,,	4	—	—	—	—	115·0	0·538	—	—	—	—	—	—	—		—

The temperature reaction starts the ninth day, shows a first fever period of about six days, and after an interval of four days, a second period of two days. A slight elevation on the twenty-second day might perhaps be a third reaction.

Piroplasms are observed from the tenth to the eighteenth day, and a second time the twenty-first and twenty-second days, that is to say, in the periods of the greatest destruction of erythrocytes. The parasites are apparently not present the nineteenth and twentieth days and, therefore, the number of erythrocytes increases.

The destruction of blood corpuscles sets in about four days after the first rise of temperature and is most intensive from the twelfth to the sixteenth day; then a slight re-creation takes place, followed by a second breaking down from the twenty-first to twenty-third day. There is even a third minimum, the twenty-ninth day.

The loss of blood cells in this case is more considerable than in 2840.

The conductivity of serum, the normal average of which is 114·7, i.e. the same as in horse 2840, varies during the attack about 7·7 per cent., and the lowest value on the twelfth day lies only 4 per cent. below the normal average.

Compared with the degree of haemolysis, the alterations of the conductivity are small. There is a precipitation on the second day, this time corresponding with an absolute decrease of the osmotic concentration (if we take the drepression of freezing point into consideration).

A slight decrease of the conductivity on the seventh, eighth, and ninth days has to be attributed to an increase of non-electrolytes (see depression of freezing point). Then follows an absolute diminution of ions (twelfth and thirteenth days) at the beginning of the blood destruction, and the minimum of conductivity coincides with the maximum of temperature elevation. With the re-increase of the number of erythrocytes (nineteenth and twentieth days) also the ion concentration of the serum becomes greater, and reaches a remarkably high value the twenty-first day.

The subsequent onset of haemolysis is again accompanied by a decrease of conductivity. A similar phenomenon can be observed in horses 2840 and 2847.

The depression of freezing point of serum after the already-mentioned jump (*vide* horse 2840) the first day, indicates a considerable increase of the osmotic pressure from the eighth to the eleventh day, that is to say, an alteration which comes in before noteworthy destructions of blood corpuscles and before the temperature rose. That this increase of the osmotic concentration is chiefly due to electrolytes is proved by the parallelism of the curves of conductivity and depression of freezing point. The subnormal value of the depression on the following days is synchronical with, and the consequence of the enormous haemolysis taking place during this time.

From the seventeenth to the twenty-fifth day there are many parallelisms between the conductivity and the crycoscopical results of serum and urine, which demonstrate the predominance of the electrolytes on the osmotic pressure of serum and prove again the regulative function of the kidneys with regard to the stability of the osmotic concentration of serum.

The conductivity of the urine which is before injection 292 × 10^{-4}, varies during the disease between 27 and 430 × 10^{-4}, or 138 per cent. ; like in horse 2840, the greatest alterations occur during the second half of the disease when there are especially two high values, the twenty-first and twenty-seventh days (both immediately before a sudden destruction of erythrocytes, or better, coinciding with a period of restitution of blood cells).

The depression of the freezing point of the urine amounts before the injection to — 2 ·2° and varies during the attack of the disease between — 0 ·94° and — 2 ·97°. The alterations correspond only in the first phase of the disease almost with those of the conductivity ; afterwards the value of the depression of freezing point remains rather constant, thus showing how the concentrations of non-electrolytes (urea ?) can vary and accomplish each other at the same time to a certain stabile osmotic pressure.

Date.	Temperature. F.		Red Blood Corpuscles.		Serum.		Morning Urine.			Afternoon Urine.			Results of Microscopical Examinations.	Pulse.
	Morning.	Evening.	Number.	Volume.	Conductivity at 25×10^{-4}	Depression of Freezing Point.	Conductivity at 25×10^{-4}	Colour.	Depression of Freezing Point.	Conductivity at 25×10^{-4}	Colour.	Depression of Freezing Point.		
1907.				%										
June 27	—	—	—	—	111·0	0·570	—	—	—	—	—	—	—	—
,, 29	—	—	—	—	113·7	0·539	—	—	—	—	—	—	—	—
July 1	99·4	100·6	—	—	113·2	0·570	351	—	2·509	—	—	—	—	—
,, 2	99·2	101·6	—	—	113·0	0·590	—	—	—	—	—	—	—	—
,, 3	99·4	99·4	—	—	115·8	0·577	362	—	2·598	—	—	—	Inj. subc. 5 c.c. blood of HF 2707	—
,, 4	99·8	101·6	—	—	113·3	0·520	—	—	—	—	—	—	—	—
,, 5	99·2	100·0	—	—	109·1	0·630	277	—	2·477	—	—	—	—	—
,, 6	99·8	100·6	—	—	116·5	0·602	—	—	—	—	—	—	—	—
,, 7	100·0	100·2	—	—	—	—	368	Clear	2·352	—	—	—	—	—
,, 8	99·6	100·6	—	42	115·0	0·629	376	Cloudy	2·795	324	Normal	2·942	—	—
,, 9	99·8	102·6	8,400,000	43	113·7	0·732	343	—	2·807	—	—	—	—	—
,, 10	100·2	102·8	8,800,000	42	114·1	—	330	Clear	2·402	—	—	—	—	—
							319	—	—	—	—	—	—	—
,, 11	100·0	101·2	9,300,000	42	108·0	0·524	372	—	2·752	100	—	—	*Piropl. equi* rare	—
,, 12	101·2	102·6	7,800,000	36	109·4	0·523	254	—	2·563	117	—	—	,, ,, ,,	—
,, 13	100·0	102·6	9,500,000	33	—	0·579	—	—	—	243	Normal	1·983	,, ,, ,,	—
,, 14	102·2	103·0	5,500,000	37	113·1	0·738	—	—	—	—	—	—	,, ,, ,,	—
,, 15	99·6	103·4	5,800,000	35	106·9	1·312	119	Light yellow, cloudy	1·461	68	Clear	1·014	,, ,, ,,	54
							112	Light brown	—	—	—	—	—	—
,, 16	100·4	102·6	5,800,000	33	109·7		40	Light yellow	1·762	—	—	—	*Piropl. equi* rare	48
,, 17	101·8	102·6	6,800,000	34	111·6	0·530	—	—	—	—	—	—	,, ,, ,,	46
,, 18	100·0	103·0	6,400,000	34	109·4		—	—	—	287	Clear	—	,, ,, ,,	50
,, 19	101·0	101·0	5,800,000	31	108·4	0·560	235	Normal	2·767	282	Normal	1·786	Mic. exam.: Negative	48
							113	Brown, clear	—	—	—	—	—	—
,, 20	101·0	102·0	5,600,000	32	110·2	0·575	12	Clear	1·817	64	Dark yellow, clear	—	Mic. exam.: Negative	42

„	21	100·8	102·0	—	—	113·4	0·534	—	—	—	165	„ thick	2·225	„ „	—
								305	Normal	—	14	Clear	—	—	—
„	22	100·6	101·8	6,000,000	35	111·0	0·529	79	Yellow, cloudy	0·548	126	Dark yellow	—	Mic. exam.: Negative	38
„	23	100·0	101·2	—	36	111·3	0·571	230	Normal	1·881	270	Normal	—	„ „	40
„	24	99·2	101·2	6,400,000	32	113·4	0·607	361	Normal	2·531	56	Clear	—	„ „	46
								224	„	—	—	—	—	—	—
„	25	99·6	101·0	—	30	110·7	0·572	228	„	1·550	241	Normal	—	*Piropl. equi* very rare	38
								204	Brown	—	—	—	—	—	—
„	26	101·2	100·2	7,000,000	33	111·2	0·556	70	Light yellow, clear	2·375	156	Dark yellow, clear	—	Mic. exam.: Negative	—
								297	Dark yellow	—	—	—	—	—	—
								27	Clear	—	—	—	—	—	—
„	27	99·8	101·4	—	33	111·8	0·543	75	Normal	2·318	211	Normal	—	Mic. exam.: Negative	—
„	28	99·4	101·4	—	—	114·8	0·588	286	Normal	2·467	—	—	—	„ „	—
„	29	99·8	101·2	7,500,000	36	112·4	0·563	300	„	2·648	—	—	—	—	—
„	30	100·4	101·6	—	—	—	0·526	195	„	1·325	331	Dark yellow	—	Mic. exam.: Negative	—
„	31	100·2	100·6	7,300,000	38	111·6	0·551	128	Clear	—	178	Normal	0·743	—	—
Aug.	1	101·0	101·4	—	—	113·1	0·568	293	Brown yellow	2·266	273	„	—	—	—
„	2	100·0	101·4	6,600,000	35	113·0	0·525	386	Normal	2·337	—	—	—	Mic. exam.: Negative	—
„	3	100·0	101·8	—	—	112·2	0·577	237	„	2·525	—	—	—	--	--
„	4	—	—	—	—	112·6	0·512	—	—	—	—	—	—	—	—

This horse (and 2848) is injected with virus from a horse foal; the *fever* begins already the sixth day. The temperature reaction lasts without interval fifteen days; it is not very pronounced, and the fever not high.

The appearance of *piroplasms*, however, is periodical; first they are seen from the eighth to the fifteenth, and secondly on the twenty-second day; hence the parasites are earlier present in the blood than in the former horses, accordingly the *destruction of erythrocytes* emphasises itself rapidly, and the first minimum of volume occurs on the tenth day. Three other minima fall on the sixteenth, twenty-second, and thirtieth day respectively.

The loss of blood corpuscles is less than in horse 2841, though there were four periods of destruction.

The conductivity of serum varies during the disease between 106 ·8 and 116 ·5 $\times$ 10^{-4} = 8 ·5 per cent. (normal 113 ·3), and the minimum lies 5 ·7 per cent. below the normal average. The conductivity falls considerably from the seventh to eighth day, then it exacerbates three times within twenty-four hours and rises again within two or three days to a somewhat higher value until it reaches, in steps, the normal.

Similarly to horse 2841, the conductivity "recovers" later, while the haemolysis continues, but still each decrease of the number of blood corpuscles causes a "relapse" of conductivity.

The depression of freezing point indicates the date of injection with a slight decrease, immediately followed by a slight increase. The beginning of the temperature reaction is marked by a rather high osmotic pressure of the serum of about ten atmospheres; the decrease both of conductivity and the number of erythrocytes is accompanied by a subnormal osmotic pressure—about seven atmospheres—after which the latter rises within three days to the enormous height of eighteen atmospheres. As this maximum almost coincides with the minimum of conductivity, it is due to accumulation of non-electrolytes in the blood.

The conductivity of the urine amounts normally to about 356 $\times$ 10^{-4}, and oscillates during the sickness from 12 to 386. It alters in the same sense with the depression of freezing point of urine. A remarkable decrease takes place on the eighth day, i.e. as soon as the haemolysis sets in.

The depression of freezing point of urine, chiefly caused by ions, shows a very low value of osmotic pressure on the twelfth day, when the osmotic pressure of serum is very high, while its ion concentration is low; that means very little electrolytes are put out with the urine and also very little non-electrolytes, though the concentration of the latter in the serum is super-normal.

Altogether the osmotic pressure shows—besides the already mentioned—two other periods of decrease which reach their minima the nineteenth and twenty-eighth day respectively.

Date.	Temperature.		Red Blood Corpuscles.		Serum.		Morning Urine.			Afternoon Urine.			Results of Microscopical Examinations.	Pulse.
	Morning.	Evening.	Number.	Volume.	Conductivity at 25×10^{-4}.	Depression of Freezing Point.	Conductivity at 25×10^{-4}.	Colour.	Depression of Freezing Point.	Conductivity at 25×10^{-4}.	Colour.	Depression of Freezing Point.		
				%										
June 27	—	—	—	—	119·2	0·590	—	—	—	—	—	—	—	—
„ 29	—	—	—	—	116·8	—	317	—	2·469	—	—	—	—	—
July 1	99·4	99·8	—	—	117·8	0·557	240	—	2·478	—	—	2·360	—	—
„ 2	99·4	99·6	—	—	116·0	0·548	243	—	2·118	—	—	—	—	—
„ 3	100·0	99·0	—	—	—	—	292	—	2·310	—	—	—	Inj. subc. 3 c.c. blood of HF 2786	—
„ 4	100·0	100·4	—	—	117·5	0·549	241	—	2·197	—	—	—	—	—
„ 5	99·6	100·4	—	—	115·8	0·572	—	—	—	—	—	—	—	—
„ 6	99·8	100·4	—	—	118·8	0·574	279	—	2·572	270	—	2·572	—	—
„ 7	100·8	99·0	—	—	—	—	—	—	—	—	—	—	—	—
„ 8	98·6	100·4	8,700,000	36	118·7	0·654	—	—	—	—	—	—	—	—
„ 9	100·0	101·6	—	33	118·4	0·734	—	—	—	—	—	—	—	—
„ 10	100·2	103·0	9,400,000	35	114·0	0·792	—	—	—	271	—	2·144	—	—
„ 11	103·8	102·2	8,700,000	33	112·5	0·729	—	—	—	280	—	2·116	—	—
„ 12	101·8	103·0	8,200,000	32	112·7	0·550	277	Cloudy	2·301	154	—	—	Mic. exam.: Negative	—
„ 13	102·0	104·8	6,700,000	31	114·4	1·307	218	Clear	1·715	—	—	—	*Piropl. equi* rare	—
„ 14	104·6	105·6	8,300,000	32	114·0	0·600	224	„	1·991	203	Dark yellow, clear	1·585	„ „ present	58
„ 15	103·0	102·0	6,600,000	30	110·0	0·533	—	—	—	85	Brown, clear	1·479	„ „ fairly frequent	56
„ 16	101·0	103·6	5,300,000	24	110·0	0·503	—	—	—	90	Red brown, clear	1·794	*Piropl. equi* present	58
„ 17	103·6	102·6	4,000,000	19	115·8	0·561	194	Red brown, clear	2·562	169	Normal	—	„ „ rare	66
							309	Normal	—	—	—	—	Conjunctiva orange	—
„ 18	100·4	101·0	6,000,000	24	112·6	0·626	161	Brown, clear	1·997	—	—	—	*Piropl. equi* rare	46
„ 19	100·0	102·0	6,800,000	31	113·1	0·517	—	—	—	99	Brownish	2·037	„ „ „	44
„ 20	101·0	104·8	5,800,000	24	110·8	0·499	170	Brownish	2·434	—	—	—	„ „ „	48

Horse 2848—(*continued*).

Date.	Temperature.		Red Blood Corpuscles.		Serum.		Morning Urine.			Afternoon Urine.			Results of Microscopical Examinations.	Pulse.
	Morning.	Evening.	Number.	Volume.	Conductivity at 77×10^{-4}	Depression of Freezing Point.	Conductivity at 77×10^{-4}	Colour.	Depression of Freezing Point.	Conductivity at 77×10^{-4}	Colour.	Depression of Freezing Point.		
				%										
July 21	105·0	103·6	—	—	112·2	0·522	—	—	—	132	Red brown	1·632	*Piropl. equi* rare Poikyl.	48
										157	Clear	—	Conjunctiva yellow	—
„ 22	103·4	103·0	3,500,000	17	110·9	0·581	143	Dark brown	—	179	Brown, clear	0·527	Poikylocytosis	58
„ 23	101·0	103·8	5,700,000	20	110·3	0·530	44	Dark yellow, clear	0·591	41	„	—	„	48
„ 24	101·2	102·0	—	19	114·1	0·563	—	—	—	162	Red brown	2·003	„	46
„ 25	99·4	101·2	4,800,000	20	112·8	0·600	164	Red brown, clear	1·536	94	Dark yellow, clear	—	„	40
							136	Red brown	—	—	—	—	—	—
„ 26	99 6	100·6	—	20	112·8	0·516	74	Clear	1·315	—	—	—	Poikylocytosis	—
„ 27	99·8	100·2	4,300,000	18	112·5	0·560	—	—	—	105	Brown	1·112	Mic. exam. : Negative	—
„ 28	99·0	100·0	—	—	114·2	0·608	215	Brownish, clear	2·026	—	—	—	—	—
„ 29	99·0	100·0	—	21	113·6	0·584	—	—	—	—	—	—	Mic. exam. : Negative	—
„ 30	99·6	100·6	5,500,000	25	116·5	0·542	—	—	—	252	Red brown	2·273	—	—
„ 31	100·0	100·4	—	—	115·4	0·541	338	Normal, thick	2·187	—	—	—	—	—
Aug. 1	100·0	100·6	5,400,000	23	115·1	0·527	—	—	—	270	Cloudy	2·394	—	—
„ 2	99·4	101·2	—	20	116·0	0·600	—	—	—	—	—	—	Mic. exam. : Negative	—
„ 3	99·8	100·0	6,500,000	23	115·5	0·537	—	—	—	332	Dark yellow	2·264	—	—
„ 4	—	—	—	—	115·2	0·526	—	—	—	—	—	—	—	—

The temperature reaction sets in on the sixth day, and shows two distinct periods of very high fever without an interval of normal temperature.

The parasites are observed from the tenth to the eighteenth day, i.e. only during the first half of the sickness, not in the second part, where there is a very high fever. Therefore the intensity of fever seems not always to be proportional to a great number of piroplasms, but they are frequent in the first part of the sickness and subsequently the *erythrocytes* suffer greatly. Their destruction begins two days after the temperature rises. It is first very slight, but then most intensive from the twelfth to the fourteenth day. A considerable reincrease of their number follows, in spite of the fact that parasites are still present. Three other periods of breaking down occur with intervals of four to five days respectively.

The recovery of the blood corpuscles is very slow and their number is, at the end of the examination time, still far from being normal.

The conductivity of serum, the average of which before the injection is 117×10^{-4}, reaches its minimum in two steps, on the seventh and twelfth day. After that the conductivity makes three attempts to get normal, but recedes each time.

The osmotic pressure of the serum begins to increase the fifth day, returns to normal, and then makes an enormous jump before the haemolysis is remarkable, and—as in horses 2840 and 2841—before conductivity has arrived at the lowest value. As the depression of freezing point shows, the osmotic pressure then oscillates periodically between $-0{\cdot}5$ and $-0{\cdot}6$.

Conductivity and osmotic pressure of urine both decrease more in the second part of the attack, both going up and down parallel with one another and the conductivity specially with the conductivity of serum, the reason of which phenomena I have already explained. The alterations of the ion concentration take place more continually and not so abruptly as in other cases.

The physical-chemical examinations on the following two horses 2961 and 2975 are made on a larger scale.

The investigations embraced :

(1) Temperature of the body.
(2) Results of microscopical and clinical examinations.
(3) Volume of erythrocytes.
(4) Conductivity of serum at 37°.
(5) Depression of freezing point of serum.
(6) Specific gravity of serum at 37°.
(7) Coefficient of thermical expansion of serum.
(8) Capillary attraction of serum in blotting paper.
(9) Quantity of serum globulines.
(10) Quantity of negative serum albumines.
(11) Quantity of daily urine.
(12) Conductivity of urine at 37°.
(13) Depression of freezing point of urine.
(14) Specific gravity of urine at 37°.
(15) Capillary attraction of urine in blotting paper.
(16) Alkalinity of urine.
(17) Quantity of water drunk daily by the animal.

After it was known that conductivity and osmotic pressure, i.e. the electrolyte and non-electrolyte concentration in serum and urine change during piroplasmosis, we expected also alterations of the specific gravity, because this is also, among other factors, dependent on the salts, and as the latter influence the surface tension, the capillary attraction should show variations as well.

The method of measuring the capillarity was that of *Goppelsroeder*, namely, stripes of blotting paper are immersed in serum or urine always to a constant depth ; the height from the level of the liquid to the upper margin of the wetted paper gives the indication of the capillarity.

This method gives not very accurate results, because the experiments were not made at constant temperatures.

The specific gravity of serum was measured twice at 25° and 37° respectively and (Pycnometer method) it was possible to calculate the *coefficient of thermic expansion* with the formula given by Kolhrausch*—

$$E = 3b \frac{p}{p^1} + \frac{1}{t^1 - t} \times \frac{p - p^1}{p^1}$$

[t—lower ; t^1—higher temperature ; p—the weight of the liquid at t ; p^1—weight of the previously at t^1 heated (pycnometer full), and then at t cooled (and contracted) liquid ; $3b$—cubic expansion coefficient of glass.]

As the expansion coefficient of water and watery solutions increases with the pressure whereunder the liquid is† and as surface pressure and internal pressure (*Tammann*) and probably imbibition pressure have principally the same effect as internal pressure, I thought that there could be some relation between coefficient of expansion and capillarity. Experience did not prove this expectation. The coefficient of expansion rather seems to go parallel with the specific gravity and adversely proportionate to the conductivity, but it shows alterations on the days of the climax.

There cannot be any doubt that the serum colloids undergo certain structural or quantitative alterations during any disease, especially in form of antibodies and their combinations with the respective antigenes.

In order to obtain the *serum globulines*, 10 c.c. of serum were dialysed against 500 c.c. of distilled water during forty-eight hours at a temperature of 1–5°. By this process the globulines precipitated ; they were separated by centrefugalisation, dried and weighed.

The quantity of negative *serum albumines* was indicated by the quantity of (positive) colloidal ferri-hydroxyd [$Fe(OH)_3$] which was necessary to produce in 2 c.c. of the (clear) residual dialysator-liquid a maximal precipitation.

The number of drops necessary for that is contained in the first column, while the second column gives the number of drops (always the same pipette used) wanted for the complete redissolution of the precipitate.

* Lehrbuch & Prakt. Physik, 10th ed. 177, 1905.
† *Chwolson*, Lehrbuch der Physik, I.

Date.	Temperature.		Serum.						Negative Serum Albumines. Number of Drops of Fe (OH), which completely		Serum Capillarity.	Urine Capillarity.	Daily Drinking Water. (Litres).		
	Morning.	Evening.	Volume of Red Blood Corpuscles.	Depression of Freezing Point.	Conductivity $\times 10^6$	Specific Gravity at 98·6.	Coefficient Thermal Expansion.	Globulines, mgr. pro. 10 c.c. Serum.	Precipitates.	Redissolves.			10 a.m.	5 p.m.	Total.
1907.			%								mm.	mm.			
Aug. 7	—	100·6	—	0·558	140·0	—	—	—	—	—	—	—	—	—	—
,, 8	100·4	101·4	—	0·551	141·7	—	—	—	—	—	61	84	—	—	—
,, 9	98·6	100·2	—	0·564	144·9	—	—	—	—	—	47	96	—	—	—
,, 10	99·8	101·2	—	0·606	143·6	—	—	—	—	—	61	81	—	—	—
,, 11	100·6	100·2	—	0·642	141·9	—	—	—	—	—	52	67	—	—	—
,, 12	99·8	100·6	—	0·631	143·5	1·0317	0·00029	—	—	—	44	75	—	—	—
,, 13	100·2	101·8	—	0·639	146·5	1·0322	0·00030	—	—	—	51	114	—	—	—
,, 14	101·0	101·0	—	0·534	138·9	1·0305	0·00031	12	—	—	50	65	—	—	—
,, 15	99·8	101·6	—	0·554	137·7	1·0293	0·00030	—	—	—	54	65	—	—	—
,, 16	100·0	101·0	—	0·559	143·2	1·0306	0·00026	12·5	—	—	45	67	20	1·5	21·5
,, 17	100·0	101·0	—	0·546	137·7	1·0311	0·00030	10·5	—	—	51	74	3·5	0	3·5
,, 18	99·8	101·4	—	0·558	139·3	1·0313	0·00030	—	2	4	51	60	16	3·5	19·5
,, 19	101·2	101·0	—	0·555	139·9	1·0299	0·00030	10	2	4	51	68	13·5	2	15·5
,, 20	99·6	101·4	—	0·546	139·5	1·0305	0·00030	14	2	3	50	62	12	1	13
,, 21	101·0	102·4	28	0·537	137·9	1·0311	0·00030	17	1	3	46	73	12·5	1	13·5
,, 22	103·0	104·0	26	0·561	140·3	1·0308	0·00030	17	1	2	46	82	18·5	1·5	20
,, 23	101·4	103·0	27	0·581	134·8	1·0320	0·00030	13	3	4	54	70	6	11·5	17·5
,, 24	103·0	105·6	25	0·510	136·0	1·0311	0·00029	11	2	4	66	60	18·5	6	24·5
,, 25	103·0	103·8	20	0·527	132·1	1·0309	0·00030	3·5	3	4	52	—	21·5	3	24·5
,, 26	102·2	102·6	18	0·527	128·8	1·0315	0·00031	18	2	3	52	56	17·5	5	22·5
,, 27	100·6	102·4	19	0·540	128·5	1·0312	0·00031	25	2	3	43	51	18	2	20
,, 28	100·0	101·8	23	0·550	135·6	1·0303	0·00030	22	2	3	43	88	20·5	4	24·5
,, 29	101·0	103·2	23	0·540	136·7	1·0296	0·00029	28	2	3	49	69	17	2·5	19·5
,, 30	102·8	101·2	25	0·556	138·7	1·0302	0·00028	29	2	3	58	98	22·5	1	23·5
,, 31	100·4	101·4	22	0·527	137·9	1·0288	—	9·5	1	2	61	114	21·5	4	25·5
Sept. 1	99·6	100·0	24	0·548	140·4	1·0280	0·00030	11	2	4	62	111	14·5	1	15·5
,, 2	99·6	100·8	25	0·550	139·6	1·0288	0·00030	8·5	3	5	62	183	5	13·5	18·5
,, 3	99·8	101·2	25	0·547	139·0	1·0294	0·00030	42	1	3	63	158	9·5	6·5	16
,, 4	100·0	103·0	23	0·548	139·1	1·0299	0·00030	14·5	2	3	55	103	11·5	4	15·5
,, 5	102·2	105·4	21	0·535	136·4	1·0298	0·00030	74·5	1	2	51	101	13	3·5	16·5
,, 6	104·4	106·2	21	0·529	136·7	1·0283	0·00032	27	2	4	63	113	12	6·5	18·5
,, 7	105·6	106·4	25	0·538	136·4	1·0281	0·00031	17·5	2	3	65	67	13·5	4·5	18·0
,, 8	104·6	—	45	0·528	138·9	1·0244	0·00031	28·5	2	4	—	—	0	—	—

Date.	Urine. Daily Quantity.	Depression of Freezing Point.	Conductivity × 10⁴	Specific Gravity.	Alkalinity.	Urine Colour.	Results of Microscopical Examinations.	Pulse.	Clinical Observations.
1907.									
Aug. 7	—	—	—	—	—	—	—	—	—
„ 8	—	2·231	327	1·031	—	Normal	—	—	—
„ 9	—	2·625	350	1·037	—	„ thick	—	—	—
„ 10	—	2·680	342	1·040	—	„ „	—	—	—
„ 11	—	2·716	243	1·038	—	„ „	—	—	—
„ 12	—	2·747	307	1·038	—	„ „	—	—	—
„ 13	—	2·306	242	1·030	—	„ „	—	—	—
„ 14	—	2·615	261	1·035	—	„ „	—	—	—
„ 15	—	2·536	233	1·031	—	„ „	—	—	Inj. subc. 3 c.c. def. blood of HF 2786.
„ 16	—	1·139	219	1·011	—	Clear	—	—	—
„ 17	10·6	1·088	178	1·011	—	Normal	—	—	—
„ 18	4·0	1·300	208	1·017	—	Muddy	—	—	—
„ 19	7·8	1·583	315	1·022	1·8	Normal	—	—	—
„ 20	5·4	1·519	297	1·020	1·5	„	—	—	—
„ 21	4·1	1·434	265	1·019	1·8	„	Mic. exam.: Negative ..	—	—
„ 22	3·9	1·255	217	1·015	1·6	„	„ „ ..	—	—
„ 23	9·3	1·520	255	1·019	1·8	Dark yellow	„ „ ..	46	—
„ 24	16·5	0·996	—	—	—	„	*Piropl. equi* rare	42	—
„ 25	15·0	0·904	174	1·009	0·9	Normal	„ „ „	70	Conj. yellow.
„ 26	18·1	0·745	108	1·006	0·4	Clear	„ „ very rare ..	70	„ „
„ 27	12·4	0·785	82	1·006	1·0	Normal	Mic. exam.: Negative ..	50	„ „ Gmelin reac. negative.
„ 28	9·1	0·945	150	1·011	1·5	„	*Piropl. equi* rare	40	„ „ „ „ „
„ 29	15·5	0·811	165	1·007	1·7	„	„ „ „	48	Gmelin reaction negative
„ 30	10·0	1·178	250	1·012	3·1	„	„ „ „	58	—
„ 31	5·8	1·274	—	1·015	3·6	„	Mic. exam.: Negative ..	58	—
Sept. 1	6·5	1·168	300	1·013	4·3	„	„ „ „	44	Normal.
„ 2	5·4	1·385	325	—	2·3	„	—	42	—
„ 3	5·7	1·458	416	1·017	3·6	„	—	—	—
„ 4	2·4	1·944	457	1·023	3·1	Dark yellow	—	—	—
„ 5	9·5	1·638	393	1·021	3·2	Normal	Mic. exam.: Negative ..	64	—
„ 6	7·1	1·348	353	1·013	4·7	„	*Piropl. equi* rare	90	—
„ 7	—	1·376	360	1·011	5·4	„	„ „ „	72	Dikkop.
„ 8	—	—	—	—	—	—	„ „ „	—	Died of horse-sickness.

The temperature rises the fifth day and shows two elevations, the first of which is the higher one. Two days after the end of the piroplasmosis the fever again sets in on account of accidental horse-sickness infection, of which the horse dies on 8th September, 1907. Like in other cases, the *parasites* are visible periodically and appear again in the horse-sickness. A phenomenon which can be observed sometimes is the breaking down of piroplasmosis immunity by an attack of horse-sickness, whereafter the animal dies of the super-position of both diseases.

The destruction of erythrocytes takes place principally in the same manner as in the foregoing horses. Clinical symptoms of *icterus* coincide with the minimal volume of the red blood cells and partially with their re-increase in number.

The osmotic concentration of serum does not increase, but rather shows only a subnormal value at the time when it has to be expected supernormal. It decreases a second time during the horse-sickness, but it is not possible to distinguish which of the diseases is the cause of this phenomenon.

The conductivity, adversely to previous cases, shows a considerable increase the first day ; then, as a rule, it goes up and down with the osmotic concentration, thus proving that the latter is chiefly due to ions, but the minimum of depression of freezing point is earlier than that of conductivity ; that means at the end of the intensive destruction of globules there is a slight increase of non-electrolytes.

Comparing the *specific gravity*, which—perhaps after previous decrease—has a slight tendency to increase, with the synchronic decrease of conductivity and number of erythrocytes and with the osmotic concentration, we must admit that the dead globules leave colloidal residues in the blood liquid whereby the resistance for the electric current, or better, for the migration of ions, is increased and an absorption of ions on these colloids takes place. Subsequently conductivity and osmotic pressure must be subnormal, while the specific gravity can increase. This idea finds a still better confirmation by the enormous divergency of the curves of conductivity and specific gravity after having passed their minimum and maximum respectively, and further, by the parallelism of the conductivity with the number of corpuscles and the depression of freezing point. The pathological colloids disappear, the serum recovers and regains the normal quantity of ions, and the blood receives a fresh supply of erythrocytes from the hematopoetic organs.

The coefficient of expansion first shows a declination the day after injection, it increases then at the same time when conductivity decreases, reaches its maximum when the conductivity is minimal, returns to the normal like the latter, and increases again at the horse-sickness attack.

The height of capillary attraction undergoes no great alterations. There is a high value on the ninth day coinciding with the minimum of depression of freezing point and the highest fever ; afterwards the capillarity becomes subnormal, increases again, and a final declination indicates the new attack. While the alterations of the capillarity have almost the same sense as those of the conductivity, the quantitative behaviour of the globulines is just reversed. This is absolutely in accordance with the experience of physical-chemistry, namely, that hydrophile colloids have the tendency to condense in surfaces and thus decrease the surface tension. At the same time we see that the colloids I mentioned above, which originate from the red globules and cause the decrease of the ion concentration and conductivity, must be to a great deal globulines.

The considerable loss of blood corpuscles—the residues of which are at least partially eliminated by liver, kidney, and spleen—involves an increase of the quantity of blood liquid in order to keep the blood pressure constant. This is—besides others (fever)—a reason for the increase of the quantity of water taken daily by the animal. This quantity increases from the second day, not continually, but with interruptions. At the same time a slight increase of the specific gravity of serum takes place, also with interruptions; that is to say, the dilutive tendency of the increased amount of liquid taken by the animal is not able to paralyse the factors which make the specific gravity higher, but on some exceptional days the latter factors are overcome by the former one, and the result is an ephemeral dilution of the serum.

But a dilution of the serum with regard to the osmotic concentration takes place all the same, as the depression of the freezing point proves. The increase of the specific gravity must be, as I said, put down to colloids.

Polyuria is a well-known symptom of piroplasmosis. In this experiment the daily quantity of urine increases roughly with the increase of the quantity of drinking water. Naturally the quantity of drinking water is always greater than the quantity of urine, but the proportions are very variable, even 15 : 1 has been observed. Therefore it is possible that the greatest quantity of body liquid is not put out with the urine. The main organs which come into consideration for the excretion of water are : (1) kidneys ; (2) lungs ; (3) skin ; (4) intestines. A small amount is lost as secretion of the lacrimal and salivary glands (and in special cases, considerable quantities of liquid leave the body in form of milk and uterine liquids).

The greater the quantity of urine, the more diluted it is, as can be seen by the comparison of its specific gravity, conductivity, and osmotic concentration with the total quantity. The conductivity decreases much more than the osmotic pressure ; that means there is specially a loss of ions more than of non-electrolytes.

It is interesting to see that at the end of the disease the conductivity is higher than normal; the depression of freezing point, however, is still subnormal. The ions are quicker regenerated than the non-electrolytes.

The capillarity of the urine goes down very slightly with the decrease of the specific gravity and then increases to supernormal height. The method permits of saying that the surface tension of urine is greater and varies much more than that of serum.

The alkalinity or the concentration of OH ions seems to decrease like that of other ions.

Horse 2961 died two hours after the blood was taken—8th September, 1907. The following are the *symptoms before death* :—The volume of blood corpuscles is enormously increased, the relative quantity of blood liquid is therefore diminished. The specific gravity of serum is very low and the conductivity slightly increased ; the serum is watery, and has lost a great deal of its colloidal components.

Horse 2975.

Date.	Temperature. F.		Volume of Red Blood Corpuscles.	Serum.				
	Morning.	Evening.		Depression of Freezing Point.	Conductivity $\times 10^4$	Specific Gravity.	Coefficient Thermal Expansion.	Globulines.
			%					
Aug. 7	—	100·2	—	0·555	150·2	—	—	—
„ 8	99·4	100·0	—	0·557	150·0	—	—	—
„ 9	98·8	100·6	—	0·545	150·3	—	—	—
„ 10	99·0	100·4	—	0·627	150·7	—	—	—
„ 11	100·2	100·4	—	0·632	148·5	—	—	—
„ 12	100·2	100·6	—	0·611	149·8	—	—	—
„ 13	99·6	101·0	—	0·596	150·0	1·0253	0·00031	—
„ 14	100·0	100·6	—	0·646	147·6	1·0259	0·00029	22
„ 15	100·2	101·8	—	0·622	145·7	1·0256	0·00029	—
„ 16	101·0	101·0	—	0·543	149·8	1·0261	0·00029	—
„ 17	100·4	104·2	—	0·549	144·7	1·0258	0·00030	—
„ 18	101·8	101·0	—	0·547	143·7	1·0255	0·00029	7
„ 19	100·4	103·0	—	0·626	145·0	1·0257	0·00030	12
„ 20	99·8	101·8	32	0·715	144·5	1·0263	0·00031	—
„ 21	100·0	101·0	30	0·540	145·6	1·0257	0·00030	16·5
„ 22	100·4	103·8	28	0·575	148·7	1·0267	0·00031	12
„ 23	102·2	103·0	30	0·538	142·7	1·0268	0·00030	19·5
„ 24	100·6	103·6	29	0·508	141·7	1·0275	0·00031	11·5
„ 25	100·4	102·2	28	0·558	137·7	1·0275	0·00030	2
„ 26	103·0	104·4	31	0·546	135·0	—	—	18·5
„ 27	101·2	101·6	26	0·532	134·2	1·0283	0·00032	4·5
„ 28	100·4	102·0	25	0·532	140·7	1·0266	0·00030	10·5
„ 29	100·6	104·6	26	0·528	138·0	1·0267	0·00029	23
„ 30	101·8	101·6	24	0·524	140·7	1·0251	0·00029	35·5
„ 31	100·6	101·0	28	0·533	146·6	1·0264	0·00029	45·5
Sept. 1	98·8	100·4	27	0·541	144·7	1·0266	0·00029	7·5
„ 2	99·2	101·0	26	0·548	144·2	1·0260	0·00029	18
„ 3	99·6	100·6	25	0·566	146·0	1·0259	0·00029	17·5
„ 4	99·8	102·0	24	0·539	145·5	1·0258	0·00030	15·5
„ 5	100·2	103·6	23	0·538	145·3	1·0255	0·00031	19·5
„ 6	100·8	103·4	22	0·550	146·6	1·0254	—	15·5
„ 7	103·2	106·4	20	0·539	142·2	1·0256	0·00028	7·5
„ 8	104·6	106·4	27	0·547	141·2	1·0265	0·00031	20·5
„ 9	103·0	102·6	25	0·538	144·2	1·0253	0·00031	23·5
„ 10	101·0	103·6	23	0·541	143·1	1·0249	0·00030	23
„ 11	101·0	101·6	23	0·532	143·8	1·0249	0·00028	30·5
„ 12	100·6	101·4	26	0·565	145·1	1·0266	0·00031	24
„ 13	100·0	100·8	24	0·582	146·0	—	—	16·5
„ 14	100·6	100·6	24	0·562	145·7	1·0275	0·00031	38·5
„ 15	100·0	100·6	23	0·573	146·3	1·0266	0·00031	37
„ 16	99·6	100·2	23	0·558	146·0	1·0272	—	81·5
„ 17	99·0	106·4?	25	0·575	147·0	—	0·00031	29
„ 18	100·2	101·4	22	0·578	147·0	1·0265	0·00031	31
„ 19	99·8	100·6	22	0·553	—	1·0263	0·00030	24·5
„ 20	100·0	100·6	25	0·561	145·6	—	—	37
„ 21	99·6	100·4	—	0·583	—	—	—	—
„ 25	—	—	25	0·543	148·7	—	—	—

HORSE 2975—(*continued*).

Date.	Negative Serum Albumines. Number of Drops of Fe (OH)$_3$ which completely		Serum Capillarity.	Urine Capillarity.	Daily Drinking Water (Litres).		
	Precipitates.	Redissolves.			10 a.m.	5 p.m.	Total.
August 7 ..	—	—	—	—	—	—	—
,, 8 ..	—	—	—	—	—	—	—
,, 9 ..	—	—	73	71	—	—	—
,, 10 ..	—	—	67	82	—	—	—
,, 11 ..	—	—	59	55	—	—	—
,, 12 ..	—	—	66	73	—	—	—
,, 13 ..	—	—	66	67	—	—	—
,, 14 ..	—	—	67	120	—	—	—
,, 15 ..	—	—	56	56	—	—	—
,, 16 ..	—	—	54	80	13	5·5	18·5
,, 17 ..	—	—	73	61	4·5	0·5	5
,, 18 ..	2	4	63	60	2	2	4
,, 19 ..	2	3	58	86	10	3	13
,, 20 ..	1	2	63	79	7	4	11
,, 21 ..	2	3	51	79	4	4·5	8·5
,, 22 ..	3	4	55	120	15	4	19
,, 23 ..	2	3	68	113	5	4	9
,, 24 ..	2	4	69	77	4·5	2	6·5
,, 25 ..	3	5 ?	67	108	4·5	3	7·5
,, 26 ..	2	4	58	91	5	5	10
,, 27 ..	4	?	50	116	6	3	9
,, 28 ..	2	4	50	94	13·5	3	16·5
,, 29 ..	2	4	53	71	5·5	2·5	8
,, 30 ..	1	3	62	71	6·5	1	7·5
,, 31 ..	1	3	69	94	23	1	24
Sept. 1 ..	3	6 ?	61	115	10	0·5	10·5
,, 2 ..	2	4	67	149	4	6	10
,, 3 ..	2	3	67	151	10	7	17
,, 4 ..	2	3	59	141	5	5	10
,, 5 ..	2	4	54	88	6·5	4	10·5
,, 6 ..	2	3	73	94	12·5	3	15·5
,, 7 ..	3	5	74	111	3·5	3	6·5
,, 8 ..	2	3	55	80	6·5	2	8·5
,, 9 ..	1	3	61	75	7·5	12·5	20
,, 10 ..	2	4	78	90	11·5	9·5	21
,, 11 ..	1	3	63	92	12·5	10·5	23
,, 12 ..	2	4	61	91	13	5	18
,, 13 ..	3	4	62	108	17	3·5	20·5
,, 14 ..	1	3	75	106	10·5	3	13·5
,, 15 ..	2	4	60	110	—	—	—
,, 16 ..	2	4	67	141	5	7	12
,, 17 ..	2	3	55	152	6	1	7
,, 18 ..	2	3	81	160	11	7·5	18·5
,, 19 ..	2	3	—	174	2	5	7
,, 20 ..	2	4	89	170	4	3	7
,, 21 ..	—	—	—	—	—	—	—
,, 25 ..	—	—	—	—	—	—	—

HORSE 2975—(*continued*).

Date.	Urine.					
	Daily Quantity.	Depression of Freezing Point.	Conductivity at $\times 10^4$.	Specific Gravity.	Alkalinity.	Coolur.
August 7	—	—	—	—	—	—
„ 8	—	—	432	—	—	—
„ 9	—	3·000	460	1·044	—	Normal, thick
„ 10	—	2·796	345	1·041	—	„ „
„ 11	—	2·642	280	1·038	—	„ „
„ 12	—	2·864	306	—	—	—
„ 13	—	2·792	331	1·041	—	Normal, thick
„ 14	—	2·635	354	1·037	—	„ „
„ 15	—	2·223	220	1·029	—	„ „
„ 16	2·3	1·852	400	1·021	—	Normal
„ 17	3·3	2·417	381	1·033	—	Dark yellow
„ 18	3·0	2·450	309	1·036	—	„ thick
„ 19	2·4	2·148	332	1·031	2·7	Normal
„ 20	1·0	2·469	438	1·035	2·2	Dark yellow
„ 21	2·4	2·726	475	1·040	2·8	„
„ 22	3·6	2·980	508	1·042	4·0	„ thick
„ 23	1·8	2·714	546	1·035	5·5	Brown yellow
„ 24	2·7	3·025	—	—	—	Dark yellow, thick
„ 25	3·4	3·403	588	1·047	7·7	Brown yellow
„ 26	9·7	1·304	306	1·013	2·5	Normal
„ 27	2·8	2·483	368	1·034	4·8	Dark yellow
„ 28	2·0	2·830	422	1·038	6·3	Brown
„ 29	2·7	2·901	375	1·038	5·4	„
„ 30	1·8	2·396	270	1·033	4·3	Dark yellow
„ 31	4·5	2·058	436	1·025	7·2	„
Sept. 1	2·4	1·795	475	1·020	7·9	Brown yellow
„ 2	1·0	2·588	580	—	5·8	Dark yellow
„ 3	1·3	2·629	566	1·036	7·2	Normal
„ 4	4·0	2·194	465	1·029	3·6	„ thick
„ 5	1·3	2·655	694	1·031	10·1	Dark yellow
„ 6	2·7	2·976	703	1·042	10·8	Brown yellow
„ 7	3·8	2·831	670	1·036	7·2	Dark yellow
„ 8	3·0	2·475	537	1·031	7·2	„
„ 9	3·8	1·733	321	1·023	4·3	Brown
„ 10	3·0	2·114	304	1·036	2·5	Brown yellow
„ 11	0·1	2·119	540	1·027	5·0	Dark yellow
„ 12	2·0	2·445	616	1·034	6·1	Normal
„ 13	1·7	2·733	596	1·045	7·2	Dark yellow
„ 14	2·7	2·578	587	1·040	7·2	Normal
„ 15	2·8	2·953	618	1·050	6·1	„ thick
„ 16	2·0	2·749	540	1·045	6·1	„
„ 17	3·4	2·520	550	1·036	5·0	„
„ 18	2·5	2·850	521	1·042	7·2	„
„ 19	2·2	2·751	518	1·044	5·4	Dark yellow
„ 20	—	2·590	470	—	4·5	—
„ 21	—	—	—	—	—	—
„ 25	—	—	—	—	—	—

HORSE 2975—(*continued*).

Date.	Results of Microscopical Examinations.	Pulse.	Clinical Observations.
Aug. 7 ..	—	—	—
„ 8 ..	—	—	—
„ 9 ..	—	—	—
„ 10 ..	—	—	—
„ 11 ..	—	—	—
„ 12 ..	—	—	—
„ 13 ..	—	—	—
„ 14 ..	—	—	—
„ 15 ..	—	—	Inj. subc. 3 c.c. def. blood DF 2564.
„ 16 ..	—	—	—
„ 17 ..	—	—	—
„ 18 ..	—	—	—
„ 19 ..	—	—	—
„ 20 ..	—	—	—
„ 21 ..	—	—	—
„ 22 ..	—	—	—
„ 23 ..	—	46	—
„ 24 ..	—	40	—
„ 25 ..	*Piropl. equi* present	36	Conj. yellow.
„ 26 ..	„ „ „	56	„ „
„ 27 ..	Mic. exam.: Negative ..	42	„ „ Gmelin reac. positive.
„ 28 ..	*Piropl. equi* rare	38	„ „ „ „ negative.
			„ „ positive.
„ 29 ..	„ „ „	50	„ „ „ „ positive.
„ 30 ..	„ „ „	46	—
„ 31 ..	—	48	—
Sept. 1 ..	—	44	Normal.
„ 2 ..	—	—	—
„ 3 ..	—	44	—
„ 4 ..	—	—	—
„ 5 ..	—	50	—
„ 6 ..	Mic. exam.: Negative ..	50	—
„ 7 ..	„ „ ..	56	—
„ 8 ..	„ „ ..	52	—
„ 9 ..	„ „ ..	—	—
„ 10 ..	„ „ ..	—	—
„ 11 ..	„ „ ..	—	—
„ 12 ..	—	—	—
„ 13 ..	—	—	—
„ 14 ..	—	—	—
„ 15 ..	—	—	—
„ 16 ..	—	—	—
„ 17 ..	—	—	—
„ 18 ..	—	—	—
„ 19 ..	—	—	—
„ 20 ..	—	—	—
„ 21 ..	—	—	—
„ 25 ..	—	—	—

The pure piroplasmosis *fever reaction* is irregular and the incubation time seems to be very short. Also this horse had an attack of horse-sickness, after having almost recovered from piroplasmosis.

Piroplasms are not frequent and are only seen for two days at the end of the first temperature reaction.

The dissolution of blood corpuscles occurs rather slowly, and—as is the rule—in steps, according to the periods of the disease. A third decrease during the horse-sickness shows that the latter is accompanied again by a piroplasmotic attack.

The osmotic pressure of serum first increases, then decreases, and remains subnormal until the end of the horse-sickness attack, with an interruption after the end of the pure piroplasmosis. Osmotic concentration and conductivity do not entirely go parallel. With regard to them and the specific gravity of the serum, the same applies, as I said, on 2961.

The coefficient of expansion shows similar alterations like in 2961.

The capillarity has two low zones, the second of which—in analogy to 2961—coincides with the minimum of conductivity and, of course, with the maximum of the expansion coefficient.

The relation between the capillarity and the *quantity of globulines* is not so distinct, like in horse 2961, and only existing during the horse-sickness reaction.

The quantity of *drinking water* differs considerably from day to day. During the piroplasmosis it is great when the conductivity of serum is high, that is to say, the organism tries to compensate excesses in the concentration of ions even when they are not exaggerated.

Polyuria cannot be observed this time, and the alterations of the urine quantity are comparatively small. The behaviour of *specific gravity, osmotic concentration, conductivity, alkalinity and capillarity* of the urine is very similar to that in horse 2961, except that, according to the absence of polyuria, the osmotic concentration and specific gravity do not decrease; conductivity and alkalinity rather increase during the horse-sickness infection; at the same time, the number of erythrocytes is at its lowest.

Of horses 3248 and 3260 we examined:—

(1) Temperature of the body.
(2) Microscopical and clinical symptoms.
(3) Volume of erythrocytes.
(4) Viscosity of blood at 37°.
(5) Surface tension of blood at 37°.
(6) Specific gravity of blood at 37°.
(7) Conductivity of serum at 37°.
(8) Viscosity of serum at 25°.
(9) Specific gravity of serum at 37°.

And of horses 3249 and 3253, besides (1)—(9):—

(10) Quantity of daily urine (filtered and not filtered).
(11) Conductivity of daily urine at 37°.
(12) Viscosity of daily urine (filtered).
(13) Surface tension of daily urine (filtered) at 37°.
(14) Specific gravity of daily urine (filtered and not filtered) at 37°.
(15) Alkalinity (filtered).
(16) Quantity of drinking water.

The surface tension is obtained by measuring the height which the blood or serum goes up at 37° in a freshly drown capillary, the diameter of which at the meniscus is determined by means of an ocular-micrometer. The surface tension is calculated by means of the formula:—

$$\text{Surface tension} = \frac{1}{2} r h s. \quad (\textit{Kohlrausch.})$$

Date.	Temperature. F.		Volume of Red Blood Corpuscles.	Blood.			Serum.				Results of Microscopical Examinations.
	Morning.	Evening.		Specific Gravity.	Viscosity.	Surface Tension.	Specific Gravity.	Viscosity.	Surface Tension.	Conductivity $\times 10^4$	
1908.			%								
Jan. 16	100·2	101·4	39	1·0578	3·46	—	1·0280	1·82	5·78	156·9	—
,, 17	101·4	100·6	—	—	3·94	—	1·0285	1·80	5·46	159·0	Inj. subc. 1 c.c. fresh blood of DF 2926.
,, 18	100·8	101·4	38	—	3·60	—	1·0266	1·80	5·76	143·8	—
,, 19	101·4	101·8	36	—	3·72	—	1·0261	1·65	5·20	151·0	—
,, 20	100·0	102·0	34	1·0517	3·50	5·26	1·0276	1·72	5·50	160·0	—
,, 21	100·6	100·6	35	1·0516	3·60	5·52	1·0260	1·75	5·58	142·3	—
,, 22	100·8	101·0	32	1·0482	2·86	5·68	1·0252	1·48	4·90	149·0	—
,, 23	100·6	101·4	34	1·0488	2·76	5·48	1·0253	1·63	4·82	149·7	—
,, 24	100·4	100·4	30	1·0490	3·12	5·50	1·0258	1·65	5·70	155·3	—
,, 25	100·4	100·8	27	1·0484	2·84	5·82	1·0252	1·55	5·38	150·8	—
,, 26	100·4	101·4	28	1·0470	2·88	5·80	1·0253	1·61	5·42	153·8	—
,, 27	102·0	102·0	32	1·0456	3·34	5·38	1·0256	1·53	4·86	151·3	Mic. exam.: Negative.
,, 28	101·0	100·8	25	1·0471	2·76	5·28	1·0256	1·62	5·46	151·6	,, ,,
,, 29	100·6	101·6	34	1·0460	3·00	5·50	1·0252	1·55	5·28	148·2	*Piropl. equi* very rare.
,, 30	101·2	101·0	27	1·0442	2·66	5·78	1·0251	1·60	5·20	152·0	,, ,, rare.
,, 31	100·2	101·0	26	1·0424	2·46	5·36	1·0251	1·63	5·84	150·8	,, ,, ,, 1 rosette.
Feb. 1	101·0	101·2	23	1·0429	2·34	5·46	1·0251	1·55	5·34	153·5	,, ,, ,, 1 ,,
,, 2	100·0	101·0	20	1·0412	2·36	5·22	1·0251	1·60	5·92	154·0	,, ,, very rare.
,, 3	100·4	100·8	20	1·0421	2·62	5·70	1·0254	1·61	5·56	154·8	Mic. exam.: Negative.
,, 4	100·0	101·0	24	1·0440	2·62	4·62	1·0259	1·64	5·70	157·8	,, ,,
,, 5	100·6	101·6	26	1·0438	2·46	5·50	1·0257	1·60	5·34	152·6	,, ,,
,, 6	100·0	101·0	28	1·0462	2·79	5·28	1·0263	1·72	5·90	157·0	,, ,,
,, 7	101·2	100·0	27	1·0467	3·14	5·38	1·0266	1·70	5·78	157·5	,, ,,
,, 8	100·6	101·0	25	1·0449	2·90	5·26	1·0260	1·72	5·50	157·0	,, ,,
,, 9	100·6	102·6	26	1·0438	2·74	4·92	1·0249	1·63	5·48	151·0	,, ,,
,, 10-15	—	—	—	—	—	—	—	—	—	—	—

The temperature reaction, starting the ninth day, is slight. *Piroplasms* are seen from the twelfth to sixteenth days ; they are rare, but in spite of that, the destruction of blood cells, beginning on the third day and showing two periods, is most abundant from the twelfth to the sixteenth day, which time would correspond with the second period of destruction.

The internal friction and *specific gravity* of the blood, as they are dominated especially by the number of blood corpuscles, also show three periods of decrease, which go almost parallel with the blood cell destruction. That these three values are not accurately going parallel is caused (1) by factors which influence only the plasma and not (or less) the corpuscles, or which influence the specific gravity in a positive and the viscosity in a negative sense, for instance, salts ; (2) by methodological and experimental errors, for instance, the specific gravity always was taken at 37°, the viscosity has been calculated for 37°, but the volume of blood corpuscles was obtained at the room temperatures. On the other hand, differences in the flow of the blood through the metal tube due to irregularities in its size and position may influence the results.

There is a specially distinct declination of specific gravity and viscosity on the fifth day, i.e. long before any clinical symptoms appear. The *surface tension* of blood does not show remarkable alterations. *The viscosity of serum* falls on the second, and again the fifth day, like the viscosity of blood ; but the minimum of the former takes place much earlier. There are many parallels with the *surface tension*. This is subnormal during the disease, but reaches the normal state again before the internal friction does.

Adversely to horse 2975, the *specific gravity* of these four horses decreases during the attack ; that means loss of colloidal or crystalloid components of serum, or of both together. In analogy to the previous six cases we should expect that the osmotic concentration decreases, and as the experiments on these four horses show also the ion concentration falls. The difference is that the conductivity alters rather early in these horses and that its alterations during the most intensive haemolysis are comparatively unimportant. As the colloidal serum components are chiefly responsible for the internal friction, we must admit that in cases of piroplasmosis, where we find a subnormal serum viscosity, loss of albuminoid substances of serum has taken place. Hence we understand that in such cases the conductivity is not considerably decreased, for these very colloids are obstacles to the migration of ions. It is remarkable that from the nineteenth day, all the methods indicate a tendency of recovery, but a few days afterwards there is a general relapse.

Horse 3260.

Date.	Temperature. F.		Volume of Red Blood Corpuscles.	Blood.			Serum.				Results of Microscopical Examinations.
	Morning.	Evening.		Specific Gravity.	Viscosity.	Surface Tension.	Specific Gravity.	Viscosity.	Surface Tension.	Conductivity × 10^4	
			%								
Jan. 16	100·0	100·4	37	1·0594	3·96	—	1·0282	1·90	5·58	157·6	—
„ 17	100·0	100·0	—	—	4·52	—	1·0276	1·95	5·54	152·4	Inj. subc. 1 c.c. fresh blood of DF 2926.
„ 18	100·6	100·6	33	—	3·50	—	1·0251	1·70	5·62	145·7	—
„ 19	100·6	101·6	36	—	3·72	—	1·0258	1·70	5·82	146·2	—
„ 20	100·0	100·6	32	1·0513	3·56	5·44	1·0252	1·70	5·64	148·5	—
„ 21	100·2	101·2	32	1·0487	3·80	5·24	1·0245	—	5·76	148·3	—
„ 22	100·2	101·0	32	1·0510	3·14	5·28	1·0256	1·62	4·88	153·3	—
„ 23	101·0	101·6	32	1·0513	3·02	5·08	1·0254	1·65	4·92	151·8	—
„ 24	100·4	101·0	31	1·0513	3·20	4·94	1·0252	1·66	5·32	157·6	—
„ 25	101·2	102·4	30	1·0508	3·24	5·64	1·0251	1·65	5·42	151·3	—
„ 26	101·8	101·4	28	1·0486	2·98	4·54	1·0253	1·68	5·42	152·0	Mic. exam.: Negative.
„ 27	100·2	100·0	34	1·0498	3·28	5·34	1·0254	1·70	5·48	152·7	*Piropl. equi* rare.
„ 28	100·4	102·0	31	1·0484	2·80	5·60	1·0255	1·78	5·28	153·0	„ „ fairly frequent.
„ 29	100·6	100·6	27	1·0438	2·66	5·38	1·0245	1·62	5·66	147·5	„ „ rare.
„ 30	99·6	101·6	28	1·0484	3·36	4·94	1·0262	1·65	5·64	155·0	Mic. exam.: Negative.
„ 31	100·6	100·2	29	1·0469	2·92	5·48	1·0255	1·68	5·20	152·7	„ „
Feb. 1	100·2	101·4	31	1·0480	3·00	5·40	1·0252	—	5·42	152·1	„ „
„ 2	100·0	101·0	33	1·0509	3·16	4·84	1·0264	1·64	5·06	154·9	„ „
„ 3	100·2	101·0	29	1·0494	—	5·40	1·0260	1·65	5·38	157·2	„ „
„ 4	100·0	100·8	26	1·0478	2·80	5·24	1·0256	1·65	5·64	150·2	„ „
„ 5	100·0	102·0	32	1·0456	2·74	5·58	1·0247	1·50	5·62	153·8	„ „
„ 6	100·2	101·0	36	1·0477	2·74	5·48	1·0257	1·70	4·64	155·0	*Piropl. equi* rare.
„ 7	99·2	100·8	28	1·0459	2·96	5·76	1·0257	1·72	5·62	157·3	Mic. exam.: Negative.
„ 8	100·6	100·2	27	1·0451	2·82	4·60	1·0254	1·65	5·38	153·8	„ „
„ 9	100·6	102·4	29	1·0450	3·00	5·40	1·0253	1·70	5·58	153·3	„ „
Feb. 20	—	—	27	1·0432	—	—	1·0241	—	—	146·2	—
„ 25	—	—	29	1·0456	—	—	1·0245	—	—	151·1	—
Mar. 2	—	—	32	1·0471	—	—	1·0253	—	—	152·1	—
„ 11	—	—	31	1·0473	—	—	1·0253	—	—	148·8	—

The temperature reaction is irregular and slight, as in the previous horse. *Piroplasms* are seen several times, viz., the tenth, twelfth, and the twentieth day.

The destruction of blood corpuscles is not very intensive, but periods are recognisable. Minima of the volume of corpuscles, viscosity, and specific gravity of the blood are noted on the twelfth and twenty-second, and of specific gravity and viscosity also on the nineteenth day.

The behaviour of *viscosity, specific gravity, conductivity, and surface tension* of serum is similar to that in horse 3248; surface tension and specific gravity show alterations in opposite sense to each other, and with the latter the internal friction goes up and down; the surface tension very often is high when the viscosity is low and vice versa, except from the fourth to the tenth day. The explanation for these phenomena can only be given when the influence of each serum component—crystalloidal, as well as colloidal—on the internal friction and surface tension have thoroughly been studied.

Date.	Temperature. F.		Volume of Red Blood Corpuscles.	Blood.			Serum.				Urine.					
	Morning.	Evening.		Specific Gravity.	Viscosity.	Surface Tension.	Specific Gravity.	Viscosity.	Surface Tension.	Conductivity $\times 10^{-4}$.	Daily Quantity.	Specific Gravity.	Filtered Specific Gravity.	Conductivity $\times 10^{-4}$.	Filtered Conductivity $\times 10^{-4}$.	Filtered Alkalinity.
			%													
Jan. 16	100·4	100·0	37	1·0605	4·32	—	1·0290	1·87	5·08	156·5	—	—	—	—	—	—
,, 17	100·2	100·4	—	—	4·66	—	1·0296	1·95	5·56	157·3	—	—	—	—	—	—
,, 18	99·6	102·4	46	—	4·68	—	1·0282	1·81	5·36	149·2	—	—	—	—	—	—
,, 19	99·6	100·8	36	—	3·60	—	1·0256	1·70	5·68	148·5	3·7	1·029	—	391	—	5·4
,, 20	100·4	101·2	34	1·0513	3·36	5·58	1·0260	1·70	5·54	146·8	5·0	1·014	—	405	—	5·1
,, 21	100·0	100·4	32	1·0497	3·32	5·60	1·0257	1·80	4·74	148·2	5·2	1·011	—	358	—	4·1
,, 22	100·2	101·8	35	1·0519	3·32	5·74	1·0261	1·65	5·12	149·2	2·0	1·044	—	736	—	9·5
,, 23	100·0	101·4	36	1·0533	3·32	5·44	1·0262	1·68	5·24	151·0	10·3	1·017	—	434	—	4·5
,, 24	100·6	100·6	33	1·0526	3·48	4·64	1·0268	1·75	5·58	153·3	3·0	1·041	1·033	669	650	5·8
,, 25	100·2	100·6	37	1·0557	3·82	5·74	1·0262	1·68	5·76	152·0	3·7	1·032	1·029	636	617	7·3
,, 26	100·6	100·4	38	1·0556	3·84	5·46	1·0265	1·75	5·66	150·7	2·6	1·044	1·038	784	758	8·7
,, 27	100·2	101·0	39	1·0529	3·74	5·50	1·0264	1·69	5·50	151·1	3·6	1·039	1·035	694	660	8·9
,, 28	100·6	102·8	36	1·0509	3·26	5·72	1·0261	1·62	5·24	150·3	7·0	1·021	1·018	519	511	5·3
,, 29	100·6	101·8	41	1·0527	3·68	5·56	1·0266	1·70	5·36	147·3	4·5	1·019	1·017	454	453	6·0
,, 30	100·4	100·8	41	1·0545	4·14	5·44	1·0272	1·75	6·40	148·3	3·6	1·024	1·021	515	501	6·7
,, 31	100·6	100·8	32	1·0493	3·38	5·50	1·0264	1·70	5·00	150·3	5·4	1·019	1·017	410	405	4·4
Feb. 1	101·0	100·6	34	1·0509	3·28	5·50	1·0266	1·70	5·46	149·5	3·6	1·029	1·025	610	576	8·1
,, 2	100·0	100·8	33	1·0510	3·20	5·50	1·0263	1·68	5·22	150·7	3·9	1·028	1·026	680	674	7·2
,, 3	100·2	100·8	32	1·0505	3·80	5·52	1·0263	1·63	5·60	148·7	8·8	1·014	1·012	362	360	4·1
,, 4	100·6	104·0	28	1·0499	3·16	5·60	1·0267	1·70	5·74	150·6	9·3	1·020	1·017	509	493	6·0
,, 5	103·6	103·4	30	1·0480	3·00	5·36	1·0266	1·70	5·64	146·2	5·6	1·017	1·015	469	462	6·3
,, 6	102·4	103·0	26	1·0448	2·62	5·56	1·0255	1·72	5·32	149·5	4·1	1·032	1·028	485	462	7·2
,, 7	103·6	103·6	21	1·0434	2·86	5·50	1·0254	1·72	5·36	146·2	7·8	1·020	1·018	527	508	7·4
,, 8	102·2	102·2	18	1·0412	2·78	5·56	1·0250	1·95	5·46	148·8	11·0	1·012	1·010	402	397	4·9
,, 9	100·4	101·6	21	1·0427	2·82	5·44	1·0248	1·75	5·38	150·7	8·5	1·010	1·007	350	339	4·3
,, 10	100·0	101·6	26	1·0466	3·56	5·10	1·0256	1·78	4·92	149·6	3·6 ?	1·020	1·017	531	522	5·4
,, 11	100·6	100·6	26	1·0472	3·26	4·98	1·0257	1·70	5·06	148·8	6·2	1·018	1·015	482	477	4·8
,, 12	100·6	100·6	24	1·0454	2·82	4·00	1·0250	1·68	4·42	150·2	6·8	1·023	1·020	538	534	5·9
,, 13	—	—	20	1·0426	2·64	5·80	1·0253	1·70	—	151·4	5·3	1·023	1·019	536	530	5·2

Horse 3249—(*continued*).

Date.	Urine—(*continued*). Solids (Sp. Gr. — 1) × Quantity.	Filtered (Sp. Gr. — 1) × Quantity.	Filtered Surf. Tens.	Filtered Viscosity.	Room Temperature 12 noon. F.	Drinking Water. (Litres.) 10 a.m.	5 p.m.	Total.	Urine—Colour. Not Filtered.	Filtered.	Results of Microscopical Examinations.
					°						
Jan. 16	—	—	—	—	—	—	—	—	—	—	Inj. subc. 1 c.c. fresh blood of DF 2926.
„ 17	—	—	—	—	—	—	—	—	—	—	—
„ 18	—	—	—	—	—	—	—	—	—	—	—
„ 19	108	—	6·34	1·20	90	—	—	—	Normal	Light brown	—
„ 20	70	—	6·54	1·14	88	13	2	15	„	„	—
„ 21	58	—	6·62	1·10	90	10	4	14	„	„	—
„ 22	88	—	7·06	1·30	76	3	3·5	6·5	„ thick	Red brown	—
„ 23	176	—	6·28	1·10	76	12	3	15	„	Red	—
„ 24	124	100	6·00	1·20	83	20	6	26	„ thick	„	—
„ 25	118	108	6·30	1·18	83	21·5	5	26·5	„	Reddish	—
„ 26	114	98	6·06	1·27	83	12	2	14	„ thick	Red	—
„ 27	140	126	6·28	—	76	5·5	3·5	9	„	„	Mic. Exam.: Negative.
„ 28	148	126	6·48	1·10	83	18	5·5	23·5	„	Red brown	*Piropl. equi* rare.
„ 29	86	76	6·32	1·12	83	4·5	2	6·5	„	Reddish brown	„ „ „
„ 30	86	76	6·34	1·16	86	10	3·5	13·5	„	Red	„ „ „
„ 31	102	92	6·44	1·11	86	14	4	18	„	Yellow brown	„ „ very rare.
Feb. 1	104	90	6·28	1·20	74	4·5	5·5	10	„	Dark red	„ „ „
„ 2	110	102	6·18	1·13	77	13	4	17	„	Red brown	„ „ „
„ 3	124	106	6·92	1·05	81	12·5	4·5	17	„	Yellow brown	„ „ „
„ 4	186	158	6·20	1·11	85	2·5	5	7·5	„	„	„ „ fairly frequent.
„ 5	96	84	6·28	1·11	76	10	3	13	„	„	„ „ „ „
„ 6	132	118	6·30	1·20	77	9	4·5	13·5	„ thick	Red	„ „ frequent.
„ 7	156	140	6·56	1·10	81	11·5	3	14·5	„	Light brown red	„ „ rare.
„ 8	132	110	6·82	1·12	85	13·5	3	16·5	„	Light brown	Mic. Exam.: Negative.
„ 9	86	60	6·72	1·10	85	5·5	6	11·5	„	Brown	„ „
„ 10	72	62	6·40	1·20	88	14·5	7	21·5	„	„	*Piropl. equi* very rare.
„ 11	112	94	6·64	1·12	86	12	6·5	18·5	„	Dark yellow	Mic. Exam.: Negative.
„ 12	156	136	6·08	1·15	86	12	5	17	„	Yellow brown	„ „
„ 13	122	100	6·38	1·18	86	11	4	15	„	Yellow	—

There is a very distinct *temperature reaction*, which seems to be the third, starting on the eighteenth day ; though *piroplasms* are present from the eleventh to the twenty-fourth day (except two days). Two long periods of haemolysis are distinguishable ; the first starting the second day—a very slight one ; the second from the thirteenth to the twenty-second day, during which considerable loss of erythrocytes takes place. This loss corresponds with the number of parasites.

Notwithstanding a strong temperature reaction, as compared with other cases, *specific gravity and viscosity* of blood behave in the same way as 3248 and 3260 and corresponds to the volume of globules. *Viscosity and specific gravity* of serum both fall on the second day, a phenomenon which has to be attributed to a diminution of serum—colloids and ions. (Compare Conductivity.) From the fourth to the eleventh day, specific gravity, surface tension, and conductivity all increase first and then decrease, but only a little, so that the final values are still higher than the first ones. The viscosity increases and decreases similarly, but the decrease is more intensive. These phenomena have to be explained by alterations of the concentration of ions which influence surface tension, viscosity, and conductivity similarly, according to their physical-chemical properties, and the specific gravity by their absolute quantity. The intensive haemolysis, beginning the fourteenth day causes also other physical conditions in the serum. Surface tension and viscosity alter reversely. Their curves, first convergent, diverge towards the end of our examination. This would point to an increase of colloidal substances, but as the specific gravity decreases, there must be a special kind of ions influencing viscosity and surface tension in reverse directions. In all probability it is a diminution of the OH ion concentration which produces the phenomeneon, for we know from numerous experiments that in piroplasmosis the alkalinity of serum is subnormal, and on the other hand, it has been demonstrated that loss of OH ions can cause a decrease of surface tension and an increase of the viscosity at the same time. (Compare chapter on Surface Tension.)

The daily quantity of urine does not show remarkable alterations—except on the sixth day—until the destruction of blood corpuscles begins, then it increases and is highest when the volume of erythrocytes is lowest. The greater the quantity of urine, the lower its *specific gravity*. As the latter is almost exclusively due to crystalloids, and among these the proportion of electrolytes remains almost constant in this case, the conductivity decreases and increases with the specific gravity.

The concentration of the hydroxyl ions is going parallel with that of the other ions. Surface tension and viscosity of the urine are altering in reverse sense, the more crystalloids are dissolved in the urine the higher the viscosity, the lower the surface tension. Certain ions influencing surface tension in a negative, viscosity in a positive, sense must have partially disappeared. As a consequence we should expect in this special case during the destruction of blood cells : the greater the urine quantity, the smaller the viscosity the greater the surface tension ; but, unfortunately, we have no normal values for comparison. By multiplying $S - 1$ (specific gravity of urine minus specific gravity of H_2O) with the total quantity of urine, we obtain with some approximation the weight of the dry residuals of the urine. In this way we see that the greater the quantity of urine, the more solid substances are put out in it, or perhaps, more precisely, the more solids the organism has to eliminate through the kidneys, the more water is required for this process. As a matter of fact, the quantity of urine and the amount of solid substances in it are likewise increased in the periods of the most intensive haemolysis.

Horse 3253.

Date.	Temperature. F.		Blood.				Serum.				Urine.					
	Morning.	Evening.	Volume of Red Blood Corpuscles.	Specific Gravity.	Viscosity.	Surface Tension.	Specific Gravity.	Viscosity.	Surface Tension.	Conductivity $\times 10^{-4}$.	Daily Quantity.	Specific Gravity.	Filtered Specific Gravity.	Conductivity $\times 10^{-4}$.	Filtered Conductivity $\times 10^{-4}$.	Filtered Alkalinity.
			%													
Jan. 16	100·6	101·8	34	1·0566	4·14	—	1·0297	1·90	5·16	155·7	—	—	—	—	—	—
„ 17	99·6	100·6	—	—	3·64	—	1·0280	1·77	5·24	149·5	—	—	—	—	—	—
„ 18	99·8	101·0	40	—	4·16	—	1·0281	1·98	5·76	149·0	—	—	—	—	—	—
„ 19	100·6	101·8	31	—	3·40	—	1·0266	1·75	5·72	149·6	3·0	1·028	—	569	—	6·5
„ 20	101·0	101·2	34	1·0528	3·74	5·36	1·0274	1·85	5·34	146·4	9·3	1·013	—	396	—	4·3
„ 21	101·0	100·8	30	1·0492	3·40	5·36	1·0265	1·78	4·54	148·9	6·0	1·011	—	341	—	3·9
„ 22	100·4	101·0	33	1·0513	3·22	4·82	1·0268	1·70	9·60 ?	149·6	4·9	1·022	—	441	—	5·5
„ 23	100·6	100·6	34	1·0527	3·36	4·82	1·0270	1·70	4·40	151·3	5·2	1·016	—	415	—	3·8
„ 24	100·6	101·8	30	1·0495	3·10	5·66	1·0261	1·63	4·54	153·1	3·8	1·029	1·026	600	582	5·5
„ 25	100·6	101·4	31	1·0501	3·24	5·56	1·0260	1·65	5·40	152·0	4·5	1·039	1·035	709	665	6·3
„ 26	102·4	102·6	30	1·0480	2·98	5·58	1·0260	1·68	5·22	149·0	12·0	1·018	1·015	470	462	4·6
„ 27	100·6	99·8	27	1·0473	2·82	4·80	1·0262	1·70	5·56	148·8	4·8	1·030	1·025	556	535	6·2
„ 28	99·8	102·0	30	1·0501	3·30	5·38	1·0268	1·68	4·88	152·5	7·2	1·015	1·013	386	384	4·4
„ 29	101·6	102·0	27	1·0458	2·94	5·78	1·0255	1·62	5·50	146·1	10·2	1·013	1·012	383	379	4·2
„ 30	100·6	100·6	22	1·0427	2·66	5·62	1·0259	1·66	5·50	148·5	7·8	1·016	1·014	389	385	4·9
„ 31	100·6	100·8	23	1·0439	2·64	5·44	1·0264	1·70	5·16	150·0	5·8	1·018	1·015	396	391	3·9
Feb. 1	101·0	101·2	25	1·0441	2·60	5·54	1·0262	1·66	5·16	148·8	6·7	1·014	1·012	383	378	3·5
„ 2	100·6	103·8	27	1·0464	2·90	5·50	1·0271	1·70	5·52	150·0	7·5	1·024	1·022	537	507	4·9
„ 3	105·0	101·0	22	1·0453	2·80	5·58	1·0270	1·70	5·40	145·8	12·0	1·008	1·005	251	248	2·7
„ 4	100·0	101·2	27	1·0491	3·16	5·46	1·0273	1·70	5·50	145·4	11·2	1·011	1·010	319	309	3·4
„ 5	101·0	101·6	25	1·0462	3·10	5·34	1·0273	1·62	5·38	147·7	10·6	1·012	1·011	333	320	3·5
„ 6	100·6	101·6	24	1·0434	2·52	5·10	1·0265	1·80	5·72	146·7	7·7	1·015	1·014	392	369	4·6
„ 7	100·4	100·8	23	1·0455	2·86	5·96	1·0271	1·82	5·38	147·4	9·1	1·012	1·011	338	322	3·6
„ 8	99·6	101·0	23	1·0445	2·64	5·42	1·0270	1·83	4·96	146·0	4·5	1·008	1·007	315	312	3·5
„ 9	99·6	101·4	27	1·0486	3·38	5·12	1·0287	1·90	5·50	149·6	5·1	1·024	1·022	493	451	5·5
„ 10	100·4	101·4	27	1·0472	3·16	5·50	1·0283	1·90	5·26	150·8	8·3	1·015	1·014	338	327	3·0
„ 11	99·8	101·4	25	1·0464	3·02	5·86	1·0272	1·95	5·40	150·0	6·1	1·020	1·019	471	459	4·4
„ 12	99·4	100·2	27	1·0468	3·00	5·36	1·0278	1·80	5·50	151·1	10·0	1·014	1·013	374	366	3·7
„ 13	100·0	101·0	26	1·0473	2·96	5·18	1·0276	1·80	—	146·5	7·8	1·012	1·010	330	321	2·8
„ 19	—	—	29	1·0483	—	—	1·0269	—	—	152·7	—	—	—	—	—	—
„ 24	—	—	34	1·0552	—	—	1·0303	—	—	157·0	—	—	—	—	—	—
March 4	—	—	30	1·0492	—	—	1·0265	—	—	151·6	—	—	—	—	—	—
„ 10	—	—	31	1·0498	—	—	1·0269	—	—	152·1	—	—	—	—	—	—

Date.	URINE—(*continued*).					DRINKING WATER. (Litres.)			URINE—COLOUR.		RESULTS OF MICROSCOPICAL EXAMINATIONS.
	Solids (Sp. Gr.—1) × Quantity.	Filtered (Sp. Gr.—1) × Quantity.	Filtered Surf. Tens.	Filtered Viscosity.	Room Temperature 12 noon.	10 a.m.	5 p.m.	Total.	Not Filtered.	Filtered.	
					°						
Jan. 16	—	—	—	—	—	—	—	—	—	—	—
,, 17	—	—	—	—	—	—	—	—	—	—	Inj. subc. 1 c.c. fresh blood of DF 2926.
,, 18	—	—	—	—	—	—	—	—	—	—	—
,, 19	84	—	7·22	1·20	90	—	—	—	Normal	Light brown	—
,, 20	120	—	6·66	1·13	88	20	14	34	,,	,,	—
,, 21	66	—	7·16	1·12	90	16	4	20	,,	,,	—
,, 22	108	—	6·82	1·15	76	14·5	5·5	20	,,	Light red brown	—
,, 23	84	—	7·14	1·13	76	18	5	23	,,	Light brown	—
,, 24	110	100	6·88	1·20	83	17·5	11·5	29	,,	Light red	—
,, 25	176	158	6·26	1·20	83	19·5	10	29·5	,,	,,	—
,, 26	216	180	6·74	1·10	83	12	3	15	,,	,,	Mic. exam.: Negative.
,, 27	144	120	7·26	—	76	5	5·5	10·5	,,	Light brown	*Pirop. equi* very rare.
,, 28	108	94	7·24	1·10	83	21	5	26	,,	Yellow brown	,, ,, fairly frequent; 1 rosette.
,, 29	134	122	4·78	1·12	83	7	4	11	,,	,,	,, ,, ,, ,,
,, 30	126	110	6·54	1·19	86	13	2	15	,,	,,	,, ,, rare.
,, 31	104	88	6·64	1·15	86	12	3	15	,,	,,	,, ,, very rare.
Feb. 1	94	80	6·96	1·11	74	4·5	5	9·5	,, thick	Light yellow brown	,, ,, ,,
,, 2	180	166	7·50	1·10	77	6·5	4	10·5	,,	Yellow	,, ,, ,, 1 rosette.
,, 3	96	60	7·60	1·00	81	4·5	6·5	11	,,	,,	,, ,, ,, 2 rosettes.
,, 4	124	112	6·68	1·10	85	9	5·5	14·5	,,	,,	,, ,, rare: 1 rosette.
,, 5	128	118	7·40	1·05	76	14	3	17	,,	,,	,, ,, ,,
,, 6	116	108	7·26	—	77	7	6	13	,,	,,	,, ,, ,,
,, 7	110	100	7·08	1·15	81	5·5	6	11·5	,,	,,	,, ,, very rare.
,, 8	36	32	6·56	1·15	85	5	4	9	,,	Light brown	Mic. exam.: Negative.
,, 9	122	112	7·42	1·23	85	6	2·5	8·5	,,	Brown	*Pirop. equi* very rare.
,, 10	126	116	7·00	1·17	88	13·5	7·5	21	,,	Yellow	,, ,, ,,
,, 11	122	116	6·26	1·15	86	9	6·5	15·5	,,	Brown	Mic. exam.: Negative.
,, 12	140	130	7·20	1·12	86	11·5	12·5	24	,,	Yellow	,, ,, ,,
,, 13	94	78	7·16	1·16	86	10	5·5	12·5	,,	Dark yellow brown	—
,, 19	—	—	—	—	—	—	—	—	—	—	—
,, 24	—	—	—	—	—	—	—	—	—	—	—
March 3	—	—	—	—	—	—	—	—	—	—	—
,, 10	—	—	—	—	—	—	—	—	—	—	—

The *temperature reaction* is very irregular and indistinct. The temperature is abnormal from the third to the twenty-second day. *Piroplasms* can be observed from the tenth to the twenty-fourth day. At the same time occurs the decrease of the number of erythrocytes in two periods, which also find expression in the specific gravity and internal friction of the blood.

Viscosity, specific gravity, surface tension, and conductivity of serum show many similarities with the respective values of 3249. The former two decrease very soon after injection, pass their minima the twelfth day, i.e. in the first period of blood cell destruction, and then the viscosity begins to recover while the specific gravity passes again through a minimum at the second onset of haemolysis. The rapid re-increase of the viscosity, however, might be partially caused by the second haemolysis. The *surface tension*, low for four days, finds itself within normal limits the eighth day. Like in horse 3249, the *conductivity* has a slight declination on the third day and increases slightly after that, which is the expression of the increase of certain ions, influencing viscosity and surface tension equally and at the same time. But these ions, or a part of them, disappear when the destruction of globules sets in, or others increase in concentration and the above-mentioned deductions do not hold good, for instance, for the nineteenth day, when the ion concentration is evidently low, the surface tension normal, and the viscosity very small. As in several other instances, the conductivity is also in this horse decreased by the destruction of blood corpuscles. Evident relation exists between *quantity* and, of course, of the *specific gravity* and *conductivity of urine* on one hand and the conductivity of serum on the other; again an expression of the tendency of the serum to keep the ion concentration constant. Adversely to horses 3249 and 2961, the quantity of urine is small on the twenty-second day, where there is the lowest amount of erythrocytes, it is high when the body temperature is high; but conductivity and specific gravity behave like in the above-mentioned horses. In 3249 and 3253 we can distinguish two great periods of the physical-chemical symptoms of the blood and the serum, the second of which shows more intensive alterations than the first with regard to blood. The limit lies in 3249 about on the thirteenth, in 3253 on the eleventh day. Among the symptoms of the urine there are three periods, the last of which is the most distinct as a consequence of its coincidence with the severest haemolysis.

The amount of *solids in the urine* is greatest on the ninth day, when the greatest quantity of urine is passed, afterwards—adversely to the previous horse—the solids become quantitatively higher or lower with the specific gravity.

The *viscosity of the urine* is lowest when fever and quantity of urine are highest. It increases when the number of blood corpuscles decreases, and decreases when the destruction stops, and it is in this way somewhat independent of the specific gravity. This is probably due to the appearance of colloidal substances in the urine (perhaps bile components).

Results and Conclusions.

The well-known *periodicity* of clinical symptoms in piroplasmotic infections emphasises itself also in the physical properties of blood, serum, and urine. One of the main factors of the periodical appearance, increase and decrease of the symptoms, is undoubtedly based on the life-cycle of the piroplasms. They generally show two periods of appearance. There are, however, more periods of decrease of the number of blood corpuscles, i.e. of haemolysis, mostly two to four. This latter fact finds the explanation in the processes of blood destruction and regeneration, because haemolysis caused by injection of haemolytic serum is not continuous, but broken by days of reconvalescence.

A better relation exists between the periods of the temperature elevations with the appearance of parasites. But the intensity of the fever is not at all proportional to the number of piroplasms.

The great dependence of internal friction and specific gravity of the blood upon the number of erythrocytes involves a similarity of the periods of viscosity, specific gravity, and volume of red blood corpuscles.

Once realised that piroplasms, being endoglobular parasites, kill blood corpuscles, that is to say, cause haemolysis, there are several questions arising; with regard to their whereabouts in the body of the host, and the manner in which these parasites enter the blood corpuscles, our investigations give no indications. With regard to the question as to the influence of the piroplasms on the protoplasm of the blood corpuscles, my former experiments in connection with haemolysis* lead to the conclusion that with all probability also the piroplasmotic haemolysis is in principle a membrane-reaction, permeabilisation of the outer layer of the erythrocyt for haemoglobin, caused by certain metabolic products, secretions or excretions of the parasitic protoplasm, whereafter the haemoglobin diffuses into the interglobular liquid and is no more able to act as an oxygen carrier.

Concerning the place where the destruction of the globule happens, two possibilities have to be considered:

(1) Haemolysis takes place in the blood stream.
(2) Infected and moribund blood corpuscles are retained in glands (liver, spleen), and completely decay there.

Supposing the destruction takes place entirely in the circulating blood, red stained serum should be expected, but as a matter of fact, this was comparatively rarely the case, and then only when haemoglobinuria and oliguria were observed. (Compare 3249.)

The explanation for the presence of haemoglobin in these cases has to be looked for in secondary renal affections. Therefore I consider the main place of haemolysis in piroplasmosis not to be the circulating system, but certain glands, with greatest likelihood, liver and spleen.

As I am only to refer within these pages about my own experiments, I limit the description of processes of destruction of blood corpuscles and the further decomposition of the various components, studied specially by human medical scientists† in the following sketch. For more particulars on the subject, see my article "Haemolysis in practical veterinary science in South Africa."‡

From this table we can see what substances temporarily can be mixed with the serum, and which cause partially the secondary physical-chemical symptoms of piroplasmosis—the sequels of haemolysis. These substances are—

Components of the stroma—colloids, electrolytes, non-electrolytes.
Haemoglobin.
Bile pigments.
Bile acids, or better, their salts.

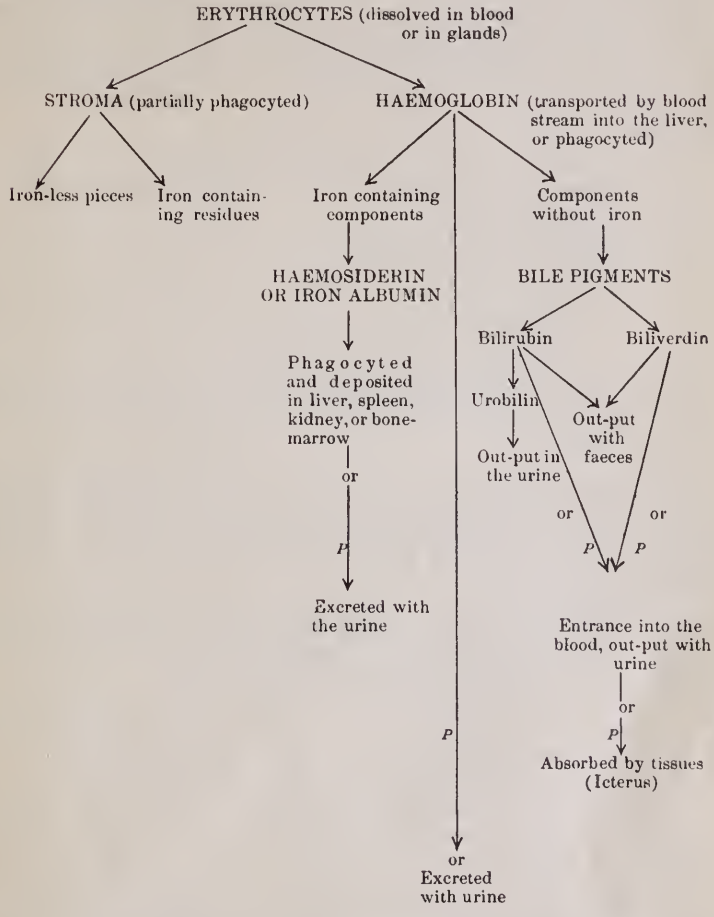
It is evident that these substances are able to alter the physical-chemical properties of serum; for instance, osmotic concentration, conductivity, viscosity, surface tension, specific gravity. As these substances are abnormal for the serum, and as the latter has the tendency to keep its physical state constant, the former are eliminated, and the consequences are alterations of

* *W. Frei*, Thesis Zurich, 1906.

† Compare *Schmidt*, Lubarsch-Osbertag's Ergebu, 3, 542, 1896; *Krans*, Cidera, 3, 416, 1896; *Kretz*, Cidera, 8 T. 495, 1902.

‡ Commemorative Publication, 1909.

the urine*. On the other hand, similar alterations can be produced by the secretions of the piroplasms, or by the metabolism caused by the fever. The decrease of osmotic pressure, conductivity, viscosity, and specific gravity of serum during the time of the disease must to a certain extent be attributed merely to dilution ; for instance, a decrease of the volume of blood corpuscles within twenty-four hours from 30 per cent. to 25 per cent. means, naturally, also decrease of the absolute blood quantity and, of course, of the blood pressure, especially when sick globules are phagocyted, and thus completely disappear out of the blood. Immediately a diffusion of liquid from the tissues into the circulation systems takes place, in order to keep up the normal haemostatic pressure. The resulting want of water is covered at the next watering time, and the effect of this is dilution of the serum, for the



P—Pathological process.

* Haemolysis does also affect the respiratorie metabolism ; respiration experiments would demonstrate this. Thorough research of the faeces would ascertain the quantity of bile components that pass the intestines.

issue of colloids and osmotically active serum components is slower. Another cause of decrease of osmotic concentration of serum is, according to Hamburger,* the absorption of electrolytes by stromata after dissolution of horse blood rich in CO_2. A similar phenomenon might occur in piroplasmosis.

The osmotic pressure of serum, that normally amounts to about eight atmospheres, shows in all cases (wherever the depression of the freezing point is observed)—except one—an increase up to about eighteen atmospheres (depression of freezing point = 1·312) in the first half of the disease (horse 2847). During the second part, it is always subnormal and can go down to about seven atmospheres (depression of freezing point = 0·508, horse 2975).

While the depression of the freezing point is an expression of the concentration of the electrolytes + non-electrolytes, the conductivity is only caused by the electrolytes of the serum—mainly NaCl. But it is not simply the reciprocity of the resistance, because we have to do with a heterogenous system (compare notes on conductivity). Regarding the pathological decrease of the conductivity of serum in piroplasmosis, there come three factors into consideration :—

(1) Decrease of the absolute quantity of electrolytes.
(2) Increase of non-electrolytes (sugar, urea), which reduce the electrolytic dissociation.
(3) Influence of serum colloids—
  (*a*) increase in concentration ;
  (*b*) structural alterations in such a manner that the migration of ions is protracted ;
  (*c*) absorption of ions whereby the latter become unable to transport electricity (ion-proteid-combinations).

Ad. 1.—Besides the already mentioned dilution of the serum by entrance of water and the eventual absorption of ions by stromata, there are at present no other reasons for loss of electrolytes.

Ad. 2.—A decrease of conductivity—the depression of freezing point remaining constant—already indicates increase of non-electrolytes. Comparatively exceedingly high concentration of the latter, however, signalises itself by great depression of freezing point and causes a decrease of the dissociation and, accordingly, of the conductivity. (Compare horses 2841, 2847, 2848, and 2975.)

Ad. 3.—Increase of serum colloids is shown by increase of the specific gravity, whilst the osmotic pressure remains at the same height (or becomes smaller), and the conductivity diminishes (2961 and 2975).

Though the decrease of conductivity is synchronical with the haemolysis and, certainly to the greatest extent, caused by the latter, the degree of the former is not necessarily proportional to the intensity of the latter.

The specific gravity of serum can become increased by colloidal residues of blood corpuscles, which influence the conductivity and osmotic pressure by simple mechanical absorption of ions. Decrease of osmotic pressure and conductivity without being accompanied by rise of specific gravity would signify a loss of electrolytes and, eventually, non-electrolytes. The measurement of the specific gravity therefore explains to a certain extent the results of both other methods. The viscosity of serum decreases in every instance. The internal friction of serum is a function of concentration and structure of the

*Osmot. Druck and Ionenlehre I. 531, 1902.

serum colloids and is influenced by its crystalloid components, especially by electrolytes. Therefore, the causes of the decrease of internal friction of serum can be the following :—

(1) Decrease of serum colloids (by dilution), in which case also the specific gravity becomes smaller ; the surface tension, however, higher.

(2) Disappearance (absorption by residuals of blood corpuscles, which afterwards are kept back in liver or spleen) of ions that influence the viscosity of the serum colloids in a positive sense. The specific gravity decreases.

(3) Forthcoming or increase of ions with the faculty of diminishing the viscosity of the serum colloids.

The factors mentioned under (1) and (2) are probably active in every instance. The loss of ions [under (2)] is also responsible for the decrease of the conductivity. Reversion of these factors has, of course, increase of the viscosity as a consequence.

The *surface tension*, which should increase when colloids partially disappear out of the serum, shows in each of the four instances distinct decreases in the beginning of the disease, a phenomenon only explicable by variations (decrease) of the concentration of certain ions that influence viscosity and surface tension in the same (positive) sense. Unfortunately, the thorough study of the influence of every singular ion on the viscosity and surface tension of solutions of serum colloids has not yet been carried out, otherwise it would be possible, after investigation of serum with various methods, to signify by name the ions or groups of ions which decrease or increase.

The physical-chemical symptoms of the urine have to vary within wider limits than those of serum on account of the constancy of the latter. The kidneys, of course, are regulators for the serum. Parallelisms between osmotic pressure and conductivity of serum and urine show the dependence of the latter on the former (2840, 2848).

Normally osmotic pressure and conductivity of urine are higher than of serum, and the former also dominated by electrolytes (like in serum). Pathologically, however, the urine values can become less than the serum values. This does not always indicate the output of very small quantities of osmotically active substances, as the low values might be derived from dilution of the same quantity of solids by a great amount of urine. Polyuria, of course, is quite a common symptom of piroplasmosis (2961). That the decrease of osmotic concentration, conductivity, and specific gravity seems rather to be secondary and a consequence of the great urine quantity is shown by a case where no polyuria occurred, and these values did not decrease (2975 ; compare also 3249 and 3253).

In addition, we find alterations of the proportions of electrolytes and non-electrolytes (urea) in such a manner that the osmotic pressure remains almost constant for many days, while the conductivity varies considerably (2841).

The parallelism of alkalinity of urine with the conductivity shows that the concentration of OH ions increases and decreases with the other ions, and does not show considerable alterations during the disease. As urine is almost free from colloidal components, its specific gravity is due to crystalloids—especially electrolytes and urea—and, therefore, decreases during the disease and—by the already explained reasons—especially when the urine quantity is great.

The viscosity of urine, being only little higher than of water (only examined in two instances), seems to decrease during the attack.

Résumé.

1. Piroplasmosis of the horse is a disease with periods also pronounced by physical-chemical alterations of blood and serum.

2. The haemolysis, produced by the intra-globular parasites, has to be considered to be the cause of a great number of physical-chemical symptoms of the serum, for the latter depend in several points on the state of the blood corpuscles.

3. Volume of blood corpuscles, viscosity, and specific gravity of the blood all decrease. Never was an increase of the viscosity noticed; contrary to what is observed in horse-sickness.

4. Viscosity, specific gravity, conductivity, and surface tension of serum also decrease, the latter especially in the beginning of the disease.

The osmotic pressure of serum decreases in every instance; in four cases out of six an increase precedes, and can amount even to more than 100 per cent. The specific gravity decreases in four instances and increases in two instances.

5. Physical-chemical alterations emphasise themselves by the methods in use before the temperature starts to rise (conductivity the first day, depression of freezing point, viscosity, surface tension, and specific gravity). Therefore, if we call incubation period the time between infection and the appearance of the first signs of the disease, it would be in some of our cases of piroplasmosis not more than twenty-four hours (2840, 2841); in other cases (3260, 3248, 3249), about four days; that is to say, much shorter than when only considering the appearance of fever.

6. The physical-chemical alterations of the urine are not typical and regular, like those of serum and blood; some of them are extraordinary, all show dependence on the state of the serum and demonstrate again the regulatory functions of the kidneys.